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CONTENTS

	PAGE
PHYSIOLOGICAL REGULATION OF THE ACID-BASE BALANCE OF THE BLOOD AND SOME RELATED FUNCTIONS. *Yandell Henderson*	131
INHIBITION. *Wm. H. Howell*	161
THE FORMATION OF BILE PIGMENT. *Arnold Rice Rich*	182
THE INFLUENCE OF MUSCLE WORK ON PROTEIN METABOLISM. *E. P. Cathcart*	225
THE INHERITANCE OF ACQUIRED CHARACTERS. *J. A. Detlefsen*	244

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PHYSIOLOGICAL REVIEWS

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PHYSIOLOGICAL REGULATION OF THE ACID-BASE BALANCE OF THE BLOOD AND SOME RELATED FUNCTIONS

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Development of knowledge of the blood as a chemical system has of late been rapid and successful. So much so in fact that there seems real danger that the approximately complete solution of one-half of a great problem may be mistaken for a solution of the whole problem: the problem of the regulation of the blood. It is probable that some investigators on this topic (1), (2), (3) do not forget this limitation; but it is quite certain that others do in effect forget that the blood is a physiological fluid regulated by a living organism, and not merely a physicochemical system.

It is true that any one sample of blood, and the gases with which it is equilibrated, consitute a physicochemical system. A large number of its factors, such as chloride, alkali, CO_2, oxygen pressure, etc., are expressible in such dependence upon each other that the alteration of one, such as the oxygen pressure, shifts all of the others. In fact, once a system of this sort is postulated, such interdependence is virtually self-evident. The recent working out of its details affords a satisfactory approach to complete solution of the purely chemical aspects of the problem. All of the alterations of such a system are reversible under the influence merely of the conditions occurring in alternation as the blood passes through the lungs and tissues, and loses or gains oxygen and CO_2 respectively (4).

But not all blood is the same. For instance, the number of corpuscles and amount of hemoglobin is 25 or even 50 per cent greater in one blood than another, or even in a single person at one time (during life in the mountains) as compared with another time. At the same CO_2 and oxygen pressure the other properties would not be the same in these various bloods, but widely different. The diagrams of these

bloods cannot be superimposed. Yet all of these bloods are, properly speaking, normal, or at least necessary, for the individuals in whose vessels they flow. Even a nephritic with so-called "uncompensated acidosis" would probably be very much upset if his blood were suddenly made "normal" for a well person. The attempt to raise a low blood alkali by merely injecting sodium bicarbonate has often proved injurious.

PHYSIOLOGICAL REGULATION. Such facts as these would seem to force recognition of a physiological regulation, a more fundamental mechanism, a superintending nature or physis,[1] determining the particular physicochemical system or interior condition of which the blood should consist at the time. It is this capacity which compensates the exterior conditions, often against the direction in which the latter tend to drive the system. It is not "vitalistic," but merely a frank recognition of fact, to say that the physis, or nature of a living thing, is as much an ultimate reality, as useful and as necessary to physiology, as the properties of the chromosome are for genetics, or those of the atom for chemistry, or of the electron for physics. Doubtless each of these ultimates will be analyzed further mechanistically some day, but each is meanwhile simply assumed in its particular science. Most investigators of the acid-base balance of the blood do not, however, see regulation thus physiologically, but merely chemically. They seem to regard the body as if it were a sort of beaker, or other vessel, containing a standard blood that may be thrown out of normality by the addition of an excess of some constituent. Thus a recent reviewer (5) says in an excellent presentation of this conception: "Only one condition can be considered as normal, that in which both blood bicarbonate and pH are within normal limits."

This conception is erroneous. On the day that these words are written a party of Englishmen is engaged in an attempt to ascend Mount Everest (6). Within a month or two they have ascended from sea level to camps at 17,000 to 23,000 feet. With them are many Tibetan porters, and in neighboring monasteries are monks who were born and who spend their entire lives above 15,000 feet. The blood of all these men contains now only about two-thirds the amount of sodium bicarbonate assumed as normal in the above quotation. In the case of those at the greatest altitude it is only about half the amount

[1] The word physiology is a compound of the Greek words physis and logos. Physis expresses the capacity of the living body to cure itself, that is, to recover equilibrium, often at a new level, which an ordinary machine is rarely able to do.

at sea level. But these men are healthy and normal in any proper sense of the word. Yet if their blood were to be suddenly brought to a sea level content of bicarbonate, they would all be extremely ill. If these Englishmen when they were at sea level two months ago, and in adjustment to a normal barometer, had suddenly been elevated to the high camp on the north col of Mount Everest, they would probably have died within an hour or two. Cases are on record in which balloonists thus suddenly elevated have in fact died (7). But the men on the slopes of Mount Everest are now quite well.

RELATION OF OXYGEN TO BLOOD ALKALI. It will probably contribute to a readier understanding of the facts and physiological relations to be discussed in this paper, if the main point is stated concisely at the outset. It has not become as yet a part of the common working ideas of physiology and biochemistry, but it should. The physiological relations defined by it are not reducible as yet to processes of present day chemistry. But the evidence for the existence of these relations is decisive. The point is this: *The amount of alkali in use in the blood of a healthy individual is fundamentally regulated and determined by the pressure of oxygen in his lungs at the altitude at which he lives. In other words, the type of blood that he has in his vessels at any one time is a function of the mean barometer at the place where he is then living.* Any change in altitude of residence involves a change of oxygen pressure, either up or down. The pressure at the new home induces a slow readjustment of the blood, requiring days or weeks to become complete. This readjustment consists in establishing a new normal amount of blood alkali. We must cease thinking of altitude as abnormal. Denver, a mile above sea level, is just as normal a residence as New York. So also Tibet and Peru, two or three times as high, are normal for their people, or for you, my reader, after a residence of a month or two. A blood alkali of 40, or even 35, is just as normal for a mountain dweller as 50 or 60 is for a sailor; or as 65 or 70 would be if a man lived for a month continuously in a caisson where the air is compressed.

This relation of oxygen to blood alkali is equally important for pathological physiology. It is the key to some of the disturbances of equilibrium (especially in respect to pH) in cardiac and in renal disease. The air-hungry cardiac patient is short of oxygen and suffers virtually from mountain sickness. His blood is rich in corpuscles like a mountaineer. But in other respects his physical defect prevents his attaining complete equilibrium. He strives to compensate, but falls more or less short. On the other hand the nephritic at sea level is under a

barometric pressure which is abnormally high for his blood alkali. The oxygen pressure in his lungs is as great as in his healthy neighbor, whose blood alkali is of normal amount. It will be shown later in this review that this relatively excessive pressure of oxygen is probably the immediate cause of the low pH in his blood. In the cardio-nephritic patient one condition or the other may predominate, or they may alternate, or mingle, not allowing acclimatization, but only partial compensation.

For the healthy man the fundamental regulation in the whole field of the hemato-respiratory functions is the oxygen pressure and supply to which he has become adjusted. The cardiac, nephritic, or diabetic patient strives to adjust, but at the same time is forced out of adjustment. The processes involved are still obscure. All that current knowledge of this matter allows, is the statement of physiological relations. They are important and extensive in their facts, but like most biological and medical phenomena they are not reducible as yet to chemical fundamentals. To pretend that we have the essential point to explain a physiological condition, when we really do not understand it at all, is the surest way of prolonging our lack of understanding. We deceive ourselves when we pretend otherwise.

ERRONEOUS CONCEPTION OF ACIDOSIS. To be more specific: It is time for us to throw off an error of thirty years' standing in regard to "acidosis." Whatever this condition is, it certainly is not merely acid poisoning. A decrease of blood alkali is probably in most cases merely incidental to something more fundamental. An excess of acid is probably not even an essential, or much less a fundamental feature of it, but merely a secondary and not invariable result. In particular "uncompensated acidosis," low pH with low alkali, so far from being uncompensated in a physiological sense, is in fact probably the body's method of seeking compensation.

This amounts to saying that the pH of the blood is not an absolute constant—the body knows none such. Like body temperature, the pH of the blood has only an approximate normal. It is considerably altered from this normal under conditions of strain, such as muscular exercise and fever; and these alterations are probably advantageous.[2] We do not regard 98.6°F., or 37.5°C., as so fixed a constant that anti-

[2] The blood is far more effectively buffered against high pH (alkalosis) than it is against low pH. This is demonstrated by the forms of all the possible CO_2 dissociation curves (see figure). The physiological effects of high pH are also far more severe than those of low pH.

pyretics and ice baths are the simple, universal, and all-sufficient recourse for any and all fever. So it is is in regard to the pH of the body fluids. This so-called constant is only one more of the factors which the living system balances against others to maintain the continuity of life and to approximate as nearly as possible to health. Physiological equilibrium and the chemical conception of equilibrium are two quite different things. The one invokes energy to maintain itself, or if disturbed to recover; it makes an effort. The other in seeking balance only goes down hill dynamically. All of this should have been obvious from the beginning. Enthusiasm over the chemical relation which pH expresses has led us to forget the teachings of Claude Bernard (8) and of Haldane (9), the true creed of physiology. Physiological regulation of physiological equilibrium is the essential condition distinguishing a living from a dead system.

Errors such as this arise from the unduly great importance which a new technical conception always assumes in our minds in comparison to the vast amount of things concerning which we are still ignorant. Most reasoning in physiology, perhaps in all science, appears to be by the process which Spinoza (10) termed "Reductio ad ignorantiam." Thus we know that pH expresses the balance of acids and bases in a fluid, and we know that pH is decreased in clinical acidosis. Therefore if decreased pH is not the cause and essence of acidosis, what is? And neither the inquirer, nor the questioned, can answer, our ignorance of any alternative answers for us conventionally. And yet, as in all such questions, the universe undoubtedly holds an indefinite number of other possibilities to us as yet unknown.

Acclimatization. To return to our topic. The experience of the Mount Everest expedition indicates that a healthy man may acclimatize quite well to 23,000 feet, barometer 325 mm. There is evidence from other sources (11) to certify that a man when fully acclimatized to any altitude whatever has nearly the same plasma pH. But at different altitudes he has not the same amount of CO_2 either in his lungs or blood. It was first shown by Fitzgerald (12), with Haldane, that the alveolar CO_2 varies with altitude in the same direction as the alveolar pressure of oxygen, or nearly with the barometer. Later I pointed out (13) from data of the Pike's Peak expedition (14) that, when the same men were tested in virtually complete adjustment to altitudes from sea level to 14,000 feet, there was a very close proportionality between the oxygen and CO_2 pressures in the lungs. From these facts it follows that the blood alkali in these men varied always very nearly in proportion to the oxygen pressure. If this were not the case the pH would have had widely varying values in acclimatization to various altitudes.

It is safe to infer that it was nearly constant in the members of the Pike's Peak expedition, at Oxford and New Haven, at Colorado Springs, and after five weeks on the Peak. Thus whenever the residence is shifted up or down, the blood alkali goes slowly down or up, to a new value in a new equilibrium. This new equilibrium is then maintained as was the old, by the regulation of respiration.

HEMATO-RESPIRATORY EQUILIBRIUM. It is essential to keep clearly in mind the constituents of this physiological equilibrium. It includes a chemical equilibrium, but is much more than that. The chemical part (15) has been described so often, and is so well understood now, that it need be only briefly referred to here. But the equally important physiological part is generally misunderstood or ignored.

Chemically, it is the proportion of carbonic acid (H_2CO_3) to sodium bicarbonate, which determines the pH. If the proportion is H_2CO_3:$NaHCO_3$::1:20, or 2:40, or 3:60, or 4:80, the pH is the same. The pH is 7.3, in all these cases. This range from 20 to 80 of blood alkali is greater than actually occurs between an altitude of 23,000 feet and one as far below sea level as the valley of the Dead Sea.

Physically, the carbonic acid of the blood in this relation is determined by Henry's law. The amount of this acid in solution varies in direct proportion to the partial pressure of the gaseous CO_2 in the air of the lungs. A pressure of 20 mm. CO_2 corresponds to 1.5 volumes per cent of dissolved carbonic acid in the blood; 40 mm. pressure causes the solution of 3 volumes per cent; and 60 mm. is in equilibrium with 4.5 of dissolved acid. So in the ratio H_2CO_3:$NaHCO_3$, we could substitute the values for CO_2 pressure (multiplied by a constant) instead of the dissolved carbonic acid, H_2CO_3. This much is well understood. But the next point, at which physiological regulation enters, is equally important and equally quantitative. The relation in this case is an inverse proportion.

Physiologically, it is the volume of air breathed which represents and determines the alveolar CO_2 pressure and the carbonic acid of the blood. It is the breathing that is regulated vitally, and thus regulates the two physical and chemical quantities. The volume of the lung ventilation is the reciprocal of the concentration of CO_2 in the lung air. In principle it is simply a dilution to a greater or less extent. Thus 0.1 liter of CO_2, if diluted in 1.0 liter of air, is 10 per cent; in 2 liters of air, it is 5 per cent; and in 4 liters, 2.5 per cent. The more the ventilation, a, the lower the percentage and partial pressure, b. Thus $a = \frac{1}{b}$.

PULMONARY VENTILATION AND BLOOD ALKALI. How much air does a man breathe? If his body produces the same mass of CO_2 in its metabolism at sea level and on Pike's Peak, or the same mass of CO_2 when healthy and when nephritic, what are the relative volumes of pulmonary ventilation? A healthy man ventilates his lungs at sea level with eighteen units of air for each unit of CO_2 exhaled. This dilution gives a partial pressure of 40 mm. CO_2 in the pulmonary air, a percentage of 5.5 CO_2, and a normal pH. If now the blood alkali is decreased so that his blood contains only $\frac{2}{3}$ the normal amount, he needs only $\frac{2}{3}$ the normal amount of dissolved carbonic acid and $\frac{2}{3}$ the previous alveolar CO_2 pressure to give the normal pH. Accordingly he breathes $\frac{3}{2}$ or 1.5 times as great a volume of air as before, for each unit of CO_2 exhaled. If his blood alkali were for some reason in-

TABLE 1

Showing relations of blood alkali and partial pressures of CO_2 in lung air producing pH 7.3, and the corresponding volumes of breathing

It will be noted that the relation of volume to pressure here is reciprocal, not proportional.

Blood alkali, volumes per cent CO_2	0	7.5	15	30	45	60	75	90
Pressure of CO_2 in lung air, mm.	0	5	10	20	30	40	50	60
Respiration, per cent of resting normal at sea level	∞	800	400	200	133	100	80	66
Respiration, liters of air per minute, assuming 6 as normal at sea level	∞	48	24	12	8	6	4.8	4

creased 25 per cent above normal, that is to $\frac{5}{4}$ of the normal, the increased amount of dissolved carbonic acid and alveolar CO_2 pressure would be provided by $\frac{4}{5}$, or 80 per cent, of the sea level volume of breathing for each unit of CO_2 exhaled.

From this aspect, the amount of the blood alkali is the factor which controls respiration. In table 1 are shown various amounts of $NaHCO_3$ (expressed in volumes per cent of CO_2). Below each of these figures is given the partial pressure of CO_2 in the air of the lungs, that would maintain the proper amount of carbonic acid (H_2CO_3) in solution to balance the alkali, and give in all cases pH 7.3. In the third line are the relative volumes of ventilation necessary to dilute the CO_2 produced in the body to the concentration shown in the second line. In the fourth line it is assumed, for the sake of illustration, that the individual's

normal breathing at rest at sea level is 6 liters per minute; and to the right and left are shown the volumes corresponding to the figures above them.

But how, it may be asked, is the blood alkali run up or brought down proportionately to the oxygen pressure. In order to understand this, so far as it is understood at all, it will be necessary to give here a somewhat broader statement of the regulation of breathing than merely to say: "Respiration is regulated by CO_2;" or "It is regulated by the pH of the blood;" or "By the blood alkali;" or "By the barometer and oxygen pressure." It is in fact regulated, so far as we can now see, by all of these factors, and doubtless by many more. This is one of the topics on which there has been and still is endless controversy to show that this or that—O_2, CO_2, pH, inherent rhythmicity, and so on—is the fundamental regulator. In the writer's opinion there is no known theory of any great value on this point, because no one has yet attempted to correlate all the data of all the hemato-respiratory functions quantitatively. Merely qualitative conceptions once were useful in this field, but we are beyond that stage now.

Regulation of respiration. Nothing so ambitious as a general theory of respiration will be attempted here; but only a statement of behavior. This behavior, as expressed by the volume of air breathed, is under three controls: 1, The immediate; 2, the intermediate; and 3, the barometric.

1. The first or immediate control of the volume of air breathed each minute is the amount of CO_2, the mass or number of molecules, that metabolism and the degree of muscular activity produce at the time. If twice as much CO_2 is produced during walking as during rest in bed, the volume of air breathed is almost exactly twice as great in the one condition as in the other, as Haldane and Priestley showed (16). This relation in walking and rest holds at sea level, and equally well on Pike's Peak. It maintains a constant alveolar CO_2 at each place.

2. But the alveolar CO_2 is not at all the same for a man at rest at sea level and a month later on Pike's Peak, nor does he breathe the same volume when walking at those places. That relation has been discussed above. We saw there that the relative volumes of air breathed for a given mass of CO_2 exhaled depend upon the blood alkali. If nearly the same pH is to exist in the blood, and if the alkali of the blood on Pike's Peak is only $\frac{4}{5}$ of that at New York, then the breathing must be, and is, $\frac{5}{4}$ or 1.25 times as great at the altitude as at sea level. So it is the blood alkali which serves as the inter-

mediate control of respiration. It sets the degree to which all the CO_2 produced in the body is diluted with air by respiration before it is exhaled from the lungs. Thus for any altitude all these functions and factors are determined by the amount of the blood alkali. It controls the volume of breathing per unit mass CO_2 produced in the body, the alveolar CO_2 pressure, and the free carbonic acid of the blood.

3. The blood alkali in turn is determined in normal persons by the barometer. Thus the oxygen pressure is the fundamental control. But how? What are the processes through which this factor slowly regulates all the others? This problem will be dealt with here from a strictly behavioristic standpoint. It appears to be a relation (of the nature of a normally constant ratio) of the oxygen pressure in the lungs and arterial blood to the blood alkali, or to the concentration of bicarbonate ions [$H\overline{CO_3}$], which determines whether or not physiological equilibrium exists. This relation is disturbed by a change of altitude; for the oxygen pressure is altered. It is also disturbed by the development of heart or kidney disease and by other disorders. In these conditions the blood alkali is the factor that is forced out of adjustment. When the blood alkali is thus lowered it leaves the oxygen pressure relatively too high. It is the direction in which the balance is disturbed that determines whether renewal of equilibrium shall be sought for through a readjustment up or down of all the hemato-respiratory factors.

An unknown factor. I once applied the term "respiratory X" (17) to the unknown factor through which oxygen and alkali act. The value of this factor at any time is the resultant of the antagonistic mass actions of oxygen and alkali. But such a term as "respiratory X" is merely a confession of ignorance. It might be a substance, such as a sulphur-containing body in the blood, or something like it, or the oxidation of sugar in the tissues, or irritation of the vagus endings in the lungs, or certain cells or cell constituents in the respiratory center, its "threshold" (whatever that is materially) or a host of other things. It certainly is not pH nor hemoglobin; but whatever it is, it is as nearly a constant as pH, for at all altitudes the relation between oxygen pressure and blood alkali (or concentration of bicarbonate ions) in the blood of a man acclimatized to that altitude—be it New York or Pike's Peak—is nearly the same. The reason for denying that "respiratory X is merely oxygen want" (18), (19) is that the same pressure of oxygen acts in opposite ways according as the blood alkali is high or low. Respiratory X depresses as well as it stimulates. The oxygen pressure at Colorado Springs, about a mile above sea level, depresses the breathing of a man who has recently lived on Pike's Peak; but it stimulates his breathing if he has just come from sea level; and it neither stimulates nor

depresses his breathing after he has lived at this pressure for a time. In the first case his alkali is low, and his volume of breathing for a time is large. But the oxygen pressure, which is higher than he has been accumstomed to, gradually decreases his volume of breathing and thus increases his alkali until he is acclimatized. In the second case the conditions and processes are exactly the opposite, so his breathing is progressively increased, and this causes a decrease of alkali until acclimatization is complete. It is to be noted particularly in this illustration that the man, whose respiration is here said to be "depressed," actually breathes a larger volume than he in whom this function is here said to be "stimulated."

REGULATION OF BLOOD ALKALI BY RESPIRATION AND pH. This ratio of oxygen to alkali appears to be the relation which is primarily upset whenever a change of residence up or down is made. If the oxygen pressure is increased relatively to the alkali, or the latter is decreased relative to the oxygen, the result is a slight depression of the respiratory center and a decrease of pulmonary ventilation relative to the blood alkali. Consequently the alveolar pressure of CO_2 is more than enough to balance the blood alkali. Thus the pH is forced out of its normal value. In the parlance of today an "acidosis" (low pH) is induced, and particularly the state now called "uncompensated acidosis." But this condition tends in a normal organism to call an increased amount of alkali into the blood, to restore a normal pH; thus acclimatization to the lower altitude soon begins to develop. Finally a normal relation is reëstablished between oxygen and alkali, and between the free carbonic acid of the blood and the blood alkali; consequently the pH also again becomes normal, and the volume of pulmonary ventilation then keeps all these factors normal for the altitude.

There is much in this picture to suggest that whenever in disease, whether cardiac, renal, diabetic, or otherwise metabolic, the blood alkali is low in relation to the pressure of oxygen, it is the relatively high pressure of oxygen which causes the slightly depressed respiration, and consequently the abnormally low pH. *Low pH in a fluid buffered like blood can never be due immediately to any other cause than relatively depressed breathing.* A hypopneic pH probably always represents, both in health and in disease, an effort to call more alkali into the blood, just as occurs when a man comes down from a mountain.

An opposite set of processes is called into play when the residence is elevated. The oxygen pressure is then made relatively low as compared to the blood alkali. Accordingly, respiration is augmented so that CO_2 is blown off in slight excess (overbreathing) (20), and the pH is thus made abnormally high. This disturbance of the balance

of carbonic acid and bicarbonate in the blood induces a passage of alkali out of the blood (21), (22). Rapidly at first, then more and more slowly, these readjustments proceed, until acclimatization is attained with physiological equilibrium at the level determined fundamentally by the oxygen pressure.

LAWS OF THE HEMATO-RESPIRATORY FUNCTIONS. The relations above described are of a degree of complexity such that they are scarcely expressible completely and clearly merely in words. They require a more mathematical form of expression. The following restatement of these relations may, therefore, be found useful for intensive consideration.

I. Regulation by the alveolar carbon dioxide. The volume of pulmonary ventilation varies almost exactly in proportion to the carbon dioxide production of the body, and thus holds the pressure of carbon dioxide in the air of the lungs nearly uniform. This is the law of Haldane and Priestley and may be expressed in the roughly mathematical form:

$$\text{Pulmonary ventilation} = CO_2 \text{ produced} \times \left(\frac{100}{\text{alveolar } CO_2 \text{ pressure}} \right) \quad ..(1)$$

In this expression the pulmonary ventilation and carbon dioxide production are the variables and are measured in cubic centimeters or liters per minute, while the alveolar carbon dioxide pressure is measured in per cent of an atmosphere and is a constant for any given amount of blood alkali. The alveolar CO_2 pressure may also be expressed in the denominator in millimeters of mercury, if the mean barometric pressure, minus the pressure of water vapor in the lungs, is substituted for 100 in the numerator.

II. Regulation by blood alkali. Any amount of blood alkali needs a corresponding amount of carbonic acid to balance it so as to give a normal pH. The blood alkali thus determines the alveolar CO_2, and by inverse proportion it thus also adjusts the pulmonary ventilation. It thus sets the dilution ratio, or volume of air breathed, per unit mass carbon dioxide exhaled (as in equation 5). It follows from this that an analysis of the alveolar carbon dioxide pressure and an analysis of the blood alkali afford almost exactly the same information and in only slightly different terms. These relations are demonstrated as follows:

Since from equation (1) above

$$\text{Alveolar } CO_2 \text{ pressure} = \frac{CO_2 \text{ production}}{\text{pulmonary ventilation}} \quad \ldots\ldots\ldots\ldots(2)$$

and since the carbon dioxide dissolved in blood plasma varies with the partial pressure of carbon dioxide in the air of the lungs, that is, by the law of Henry,

$$H_2CO_3 = \text{Alveolar } CO_2 \text{ pressure} \times \text{constant} \quad \ldots\ldots\ldots\ldots(3)$$

and since respiration adjusts the relations of the dissolved to the combined carbon dioxide, so that by the law of L. J. Henderson,

$$\frac{H_2CO_3}{NaHCO_3} = \frac{\text{H-ions}}{\text{a constant}} \text{ and gives a uniform H-ion concentration} \quad \ldots(4)$$

therefore if the right hand term is constant

$$NaHCO_3 = \frac{\text{constant} \times CO_2 \text{ produced}}{\text{pulmonary ventilation}} \quad \ldots\ldots\ldots\ldots\ldots\ldots (5)$$

III. Equilibrium values of hemato-respiratory factors. The values for the arterial oxygen and CO_2 pressures, and for the amount of blood alkali have very nearly the same proportion to each other at all altitudes, while the volume of breathing per unit mass carbon dioxide produced varies inversely to the other quantities. Thus, if K_1, K_2, K_3, K_4 are constants with the values respectively of 100 mm., 50 volumes per cent, 40 mm. and a dilution factor 18, these being normal sea level values, the law for all oxygen pressures may be formulated thus:

$$\frac{\text{Oxygen pressure in lung air}}{K_1} = \frac{\text{Blood alkali}}{K_2} = \frac{\text{Alveolar } CO_2 \text{ pressure}}{K_3} = \frac{K_4}{\text{Pulmonary ventilation per unit mass } CO_2 \text{ eliminated}} \quad \ldots\ldots\ldots (6)$$

At sea level each member of this equation comes to 1/1; on the shoulder of Mount Everest each would be about 1/2.

IV. The control exerted by oxygen over respiration and thus over pH. Whenever the partial pressure of oxygen in the arterial blood (expressed in millimeters) is above the ratio 100:50, as compared with the alkali (expressed in volumes per cent combined carbon dioxide) respiration is depressed, relatively to the blood alkali, so that the ratio H_2CO_3:$NaHCO_3$, and presumably the H-ion concentration, are raised above normal (low pH). Whenever the oxygen-alkali ratio falls below 100:50, respiration is stimulated, relatively to the blood alkali. Thus the ratio H_2CO_3:$NaHCO_3$, and presumably the H-ion concentration, are decreased (high pH).

V. Regulation of the blood alkali by pH. Whenever a depression of breathing forces and holds the ratio H_2CO_3:$NaHCO_3$ above normal, causing a low pH, an increase of blood alkali tends to occur. Whenever overbreathing holds the ratio below normal, causing a high pH, alkali tends to pass out of the blood. Both processes tend thus to bring the ratio back to normal and to restore a normal pH at a new level of alkali. Thus if the arrow signifies "leads to"

$$(\text{Oxygen: } [\overline{HCO_3}] > 100:50) \rightarrow \text{Depressed breathing} \rightarrow \frac{H_2CO_3}{NaHCO_3} \text{ (above normal)} \rightarrow \text{increase of alkali and thus compensated pH with a higher blood alkali} \ldots\ldots\ldots (7)$$

$$(\text{Oxygen: } [\overline{HCO_3}] < 100:50) \rightarrow \text{Overbreathing} \rightarrow \frac{H_2CO_3}{NaHCO_3} \text{ (below normal)} \rightarrow \text{decrease of alkali and thus compensated pH with a lower blood alkali} \ldots\ldots\ldots (8)$$

This means that the immediate cause of low pH is always a relative depression of breathing, and that the purpose of low pH is to call more alkali into the blood; while the immediate cause of high pH is always overbreathing and its purpose is to drive some of the alkali out of the blood. (For a fuller statement of these relations, see the series of papers on Hemato-Respiratory Functions, by Haggard and Henderson, Journal of Biological Chemistry, Vols. 39–47, 1919–21, and also those on the Respiratory Regulation of the CO_2 Capacity of the Blood, same journal, February, 1918, Vol. 33, p. 333.)

IMMEDIATE CAUSE OF LOW pH. It may be well to explain what this discussion just here is aiming at: It is to show that acidosis, in the sense of low pH, is simply depression of breathing; and that its more fundamental causes lie therefore somewhere in the regulation of respiration. The conception is now prevalent that when the balance of acids and bases in the living body is upset, an excessive production of acids is necessarily involved. It is now generally believed that the clinical condition called "acidosis" is, therefore, essentially the same as that which can be produced by injecting acids. The best experimental parallel is that effected by J. B. S. Haldane (23), who swallowed considerable amounts of ammonium chloride. The ammonia was converted into urea and excreted, while the hydrochloric acid accumulated in the body. Consequently the sodium bicarbonate of the blood was lowered to such an extent that, as J. S. Haldane (24) (his father) recently remarked, "I was almost ashamed to walk with him at a leisurely pace along the streets of Oxford, for he was panting so hard as to attract everyone's attention."

The general conception of poisoning with acid originated long before physical chemistry had reached the point of expressing an acid-base balance by its hydrogen ion concentration (or its clumsy negative logarithm) pH. When it was found that the pH of the blood in clinical acidosis is low, the simple inference (by the reductio ad ignorantiam) was therefore that acid elements predominated in the blood. According to that view, that is virtually all there is to it. This would be the case if the blood were in a beaker, under a bell jar, in a thermostat.

But in the body in addition to structures and arrangements corresponding to the beaker, bell jar and thermostat, there is a regulating device, one of many, the respiratory center. This device normally adjusts the ventilation of the living bell jar, the alveolar air of the lungs, so that the concentration of CO_2 in the air has not a fixed absolute value, but a fixed value relative to the amount of sodium bicarbonate in the blood. The nature of the blood, both in the beaker and in the body, is such that the only free acid that can ever occur is H_2CO_3.

Its amount is directly proportional (by Henry's law) to the concentration of CO_2 in the equilibrating air. Furthermore, the pH is the immediate resultant of only two factors, $NaHCO_3$ and H_2CO_3. If both are normal, pH is normal; if both are twice normal, pH is still normal; if both are half normal, pH is nevertheless normal.

Now suppose a chemist with such a system set up in his laboratory suspected that something had happened by which a little acid had been added, or a little alkali withdrawn from the contents of the beaker. He would determine the amount of $NaHCO_3$, and perhaps find his suspicion confirmed. But he would not expect to find the pH altered. On the contrary he would expect to find the ventilation increased, so that the concentrations of CO_2 in the air and of H_2CO_3 in the blood were lowered in exact proportion to the decrease of alkali.

But suppose that he finds that the pH is in fact lowered. This is usually the case in clinical acidosis. What inference should even the chemist, or certainly a physiologist with regulation as a creed, draw from this alteration of pH? Only one conclusion is possible; namely, that the mechanism regulating the atmosphere in the bell jar has been in some manner depressed. In other words, let the fundamental cause by what it may, the only possible immediate cause of a low pH in the blood in the living body is a relative depression of breathing, with the emphasis on the word relative.

Let us see how much of a relative depression of breathing would have such an effect, and how much of an increase of ventilation would give a normal pH again. This is the more important because the ventilation is in fact continually varying with every bodily movement throughout life. Yet it is often treated, or neglected, by workers in this field, as if the volume of air breathed were almost as rigid a constant as a man's height.

QUANTITATIVE RELATION BETWEEN VOLUME OF BREATHING AND pH. It is quite easy to make this estimate by means of the CO_2 diagram introduced by Haggard and the writer (25), also independently by Straub and Meier (26) in Germany, and slightly modified by Van Slyke (27) (see fig. 1). This diagram evaluates all points in the CO_2 dissociation curve of the blood in three respects: 1, the amount of combined CO_2 and the alkali in use, in other words the $NaHCO_3$; 2, the pressure of CO_2 in millimeters and therefore the amount of carbonic acid, H_2CO_3, in solution; and 3, the relation of carbonic acid to bicarbonate, in other words, the pH. The scales for these quantities

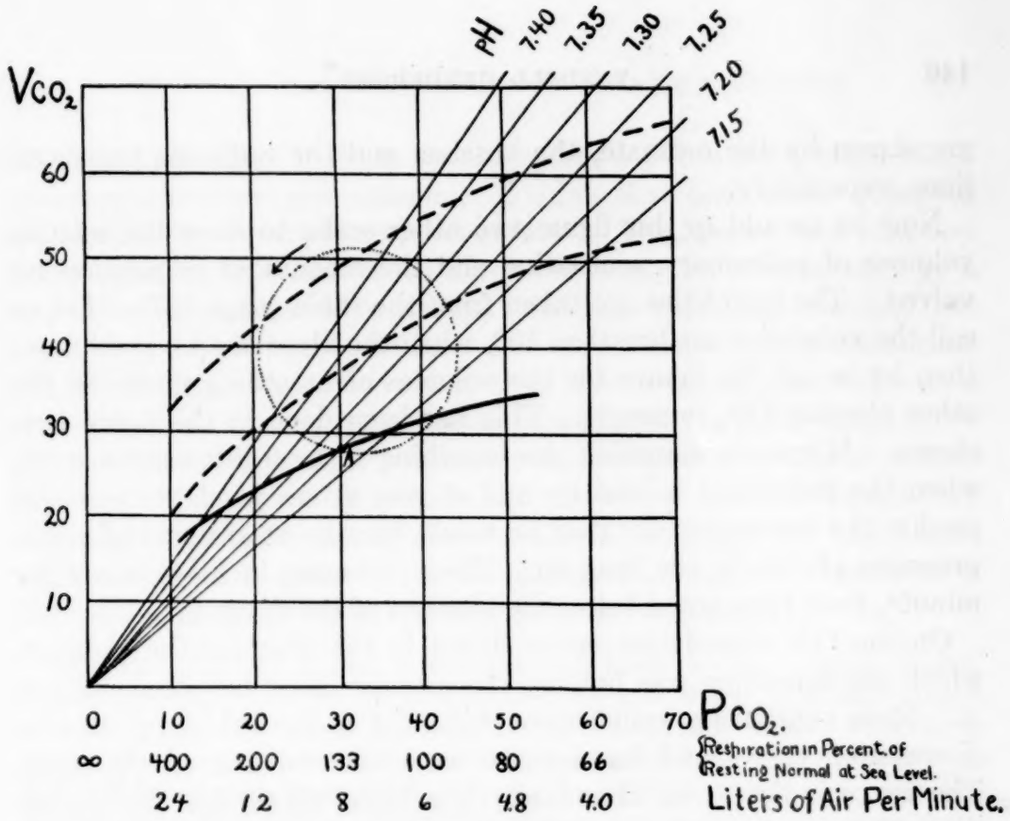

Fig. 1. *The CO_2 diagram.* The point marked *A* indicates the condition of the arterial blood. It lies upon the CO_2 dissociation curve, the solid line, which expresses (by the scale at the left) the alkali available in the blood, its CO_2 combining power, at the time. The point on the curve at which *A* is held is determined by the pressure of CO_2, according to the scale immediately below the abscissa. In the next scale below are given the volumes of breathing, and below that the number of liters of air per minute in the breathing, which would give each particular pressure of CO_2 and would hold the arterial blood, *A*, at corresponding points on the dissociation curve. The oblique lines express the pH for the points at which each intersects the dissociation curve. The broken curves indicate the limits of normal variations in the amount of blood alkali in healthy people at sea level. From this it is seen that the dissociation curve here indicated expresses a low blood alkali, and the *A* point lying on the line of pH 7.2 indicates so-called "uncompensated acidosis." If the volume of breathing were increased from 8 liters up to 12 liters, *A* would lie at the intersection of the dissociation curve and the normal pH line 7.3. The circle indicates the general character of the movement of the *A* point during acclimatization to altitude, and after return to sea level. This movement to the left and down and then to the right and up again is due both to movement of *A* along the CO_2 dissociation curve and to a rise or fall of the curve itself. It expresses the decrease of blood alkali when a man goes up to a greater altitude to live and the increase again when he returns to sea level. The rotation is counterclockwise, indicating that when breathing is stimulated by less oxygen, the *A* point is moved to the left, or alkaline side of normality, and the alkali falls, carrying the *A* point down with it. When, on the contrary, respiration is depressed by a relative excess of oxygen, as in a mountaineer who has come down to sea level or in a nephritic at sea level, the *A* point swings to the right or acid side of normality in the attempt to call an increased amount of alkali into the blood.

are shown by the ordinate, the abscissa and the radiating transverse lines respectively.

Now let us add to this figure two other scales to show the relative volumes of pulmonary ventilation and the amount of respiration involved. The quantities are taken from the table (page 137). Let us call the volume of air breathed 100, when the alveolar CO_2 is 40 mm.; then let us set the figures for the volumes of breathing under all the other alveolar CO_2 pressures. This has been done in the figure here shown. Moreover, assuming the breathing to be 6 liters per minute, when the individual is healthy and at rest at sea level, we can also predict the volumes of air that he would breathe at any and all other pressures of CO_2 in the lung air. These volumes, in liters of air per minute, have been noted below the abscissa of the diagram.

On the CO_2 dissociation curve shown in the diagram the point at which the breathing was holding the arterial blood has been marked *A*. More ventilation would move this point to the left along the CO_2 dissociation curve, and less ventilation would move it to the right. The curve indicates an abnormally low blood alkali, for the similar dotted curves above it are the limits of normal variation at sea level. At a volume of breathing of 7 liters per minute or 36 mm. CO_2 pressure, the CO_2 dissociation curve crosses (approximately) the line of pH 7.15. According to the diagram, 12 liters per minute of breathing would give an alveolar CO_2 pressure of 20 mm., and at this pressure the dissociation curve indicates that the pH of the blood would be 7.32. Actually a nephritic or diabetic with the low alkali here shown usually has a pH distinctly below normal, and his condition is termed "uncompensated acidosis." But be it noted from the diagram: he need only breathe 12 liters of air, instead of the 8 or 10 that he does breathe, to effect full compensation in respect to pH. There is no mechanical difficulty about breathing this volume; anyone who takes a walk breathes as much as that. He could, but does not; and the fact that he does not is the immediate cause of the low pH. Absolutely his breathing is much above normal, it may be 8 or 10 liters instead of 6. But relatively it is nevertheless depressed below the volume (12 liters) that it should have at the existing blood alkali.

May it not be that, his alkali having been lowered in some way, the high relation of oxygen to bicarbonate induces a relative depression of breathing? Inhalation of oxygen has this effect gradually on a normal man. On general physiological principles of regulation is it not probable also that the low pH represents an effort to call more

alkali into the blood? So to speak, he has a Pike's Peak blood alkali and a sea level oxygen pressure. I think that both these questions may be safely answered in the affirmative. The figure shows that any reasonable degree of low blood alkali can easily be compensated by a quite feasible increase of breathing. It shows that the breathing that would give the pH found clinically, and that which would give a normal pH, are not so different in volume as to involve much additional strain on respiration. Extreme conditions which involve a blood alkali below 20 are almost always rapidly fatal, for these require a volume of breathing which it is not possible to maintain indefinitely. But for all ordinary degrees of "acidosis" in the sense of low blood alkali, the immediate, sufficient and sole cause of the low pH is the (relative) depression of the respiratory center and of the volume of breathing.

Some of those who neglect respiration as a factor in this relation are led, quite unconsciously, to state impossibilities, and it is evident that most readers of the biochemical journals now see nothing incongruous in such statements. For instance, we are informed that in ether anesthesia the "excitement hyperpnea" involves a great increase of pulmonary ventilation, while the production of CO_2 may even be lessened by the anesthetic; and yet (they say) there is also a rise of CO_2 tension in the arterial blood, and this rise is a factor, along with a slightly lowered alkali, in causing the hyperpnea. A wonderful trap for the unwary is that negative logarithm of the figure obtained by dividing the blood alkali into the tension of CO_2 and multiplying by a solubility constant and then by another factor (K, supposed—perhaps erroneously—to be constant); all of which is pH. It becomes indeed the classic "pons asinorum" when we substitute for the tension of CO_2 the reciprocal value for the volume of breathing per unit mass CO_2.

But suppose we were told (e.g., by some university official) that simultaneously with a rise of prices of food and clothes, and possibly a slight decrease of professors' salaries, the said professors and their families would be able to live in greater affluence than at present. In this case the reciprocal relation of prices to affluence would not be as difficult to keep in mind as that between pulmonary ventilation and the tension of CO_2. But then we do not forget prices, as we do forget the volume of pulmonary ventilation; nor do we confuse ourselves by using the negative logarithm of the ratio of the goods on the market to the quantity of money in circulation.

In our field it would be better to use the direct figures for H-ion concentration. I would again suggest the form of expression cH7 = 0.5, or cH6 = 0.8 and so on, as a short way of writing $cH = 10^{-7} \times 0.5$, or $cH = 10^{-6} \times 0.8$ etc. This form is as short as that of pH. It means that the actual H-ion concentration, if multiplied by the power of ten indicated at the left of the equal sign, would amount to the fraction of a mol shown by the decimal at the right of the equal sign.

DEFECTS OF PRESENT THEORY. One of the striking defects of the acid poisoning theory is that blood alkali and the H-ion concentration

are not proportional functions. In most biochemical writing they are still treated as if the alkali and pH must always rise and fall together. So in 1919, Haggard and I introduced the CO_2 diagram (see the figure) for the purpose of demonstrating graphically that while blood alkali varies up and down, pH swings round on the arc of a circle. But this demonstration had little effect. The conception that acidosis is simply neutralization of bicarbonate by acid is still strongly held. A closely similar graphic expression is now usually employed as demonstrating the purely chemical basis of acidosis.

Another striking defect of the chemical theory is that in some conditions of clinical acidosis, the only evidence that the blood alkali has been neutralized by acids is that the alkali is low. Consider the nephritic type. The normal kidney has as one of its chief functions the adjustment of the osmotic pressure of the blood. The diseased kidney may not excrete chlorides as efficiently as normally, and attempts to compensate by excreting other substances instead. Some recent developments (28) suggest such a possible explanation of the low blood alkali in nephritis, which is at least as probable as the theory by which the body of the nephritic is supposed to produce an excess of acids. For the acids are undiscoverable, and a decrease of blood alkali in any other way would have the same result: namely, a call for a decrease of carbonic acid and an increase of breathing to bring it about, so as to prevent an extreme disturbance of pH. To a considerable degree the breathing is increased, but not enough to restore an entirely normal pH. The pressure of oxygen is now abnormally high in relation to the alkali, and this relation tends to depress respiration sufficiently to cause a distinctly low pH. The patient, therefore, breathes a larger volume of air than if he had a normal blood alkali, but not a sufficient volume to exactly balance the alkali. As pointed out in a previous section, he is like a man whose blood alkali is that of a great altitude, but who never succeeds in attaining complete acclimatization because the oxygen pressure is that of sea level where he lives.

The present confusion in this field results largely from the use of the word "acidosis" in several different senses: 1, the clinical condition; 2, low pH; 3, low alkali bicarbonates, or low CO_2 capacity, or low "alkaline reserve"; 4, ketosis. The word "acidosis" should be used only in the first of these senses, a symptom complex. The second should be called "hyperhydria" or "relative hypopnea"; for the latter term would indicate the immediate cause of the condition, while the expression "uncompensated acidosis," now generally used, fails to remind us what function it is (namely respiration) that is not compensating. The third is now denominated "hypocapnia" in the current German literature and

should be so called in our journals also. The use of this term should indicate merely that the CO_2 of the blood, that is, the bicarbonate content, is low; but with no implication as to how this condition is caused in the particular case; so that it may not be confused with a simple acapnia from overbreathing. The various senses of "alkalosis," as the term is now used, would then be denominated "hypohydria," for high pH, and "hypercapnia" for high alkali bicarbonates.

The theory of acid poisoning as popularly used rests largely upon the term "alkaline reserve (29)." This expression served at first a very useful purpose in calling attention to an important and measurable property of the blood. But it almost deserves to be classed with "phlogiston" in the extent to which it has misled thought. It is only just to add that Van Slyke, who introduced this term, has done more than anyone else to demonstrate that by far the greater part of the acid neutralizing power of the blood, the true alkaline reserve, is really afforded by the hemoglobin alkali of the corpuscles, and relatively little by the sodium bicarbonate of the plasma.

So long as the expression alkaline reserve is used to denote bicarbonates it must continue to mislead by suggesting that it is the $NaHCO_3$ of the plasma which neutralizes any acid administered by mouth or intravenously or that may be formed by some perversion of metabolism. Araki (30) found many years ago that after asphyxia, especially from carbon monoxide, lactic acid occurs in the blood and urine. It was, of course, wholly in the form of sodium lactate, for the free acid in appreciable quantities can never occur in a system buffered like blood. The Hill-Meyerhof (31) conception of muscular contraction makes lactic acid the essential factor in every bodily movement, and suggests an escape of the excess from the muscles into the blood during intense exertion. This escape has been verified by Barr, Himwich and Green (32). They have shown that the blood alkali is distinctly decreased for some time after strenuous muscular work. It has seemed logical to determine the amount of this decrease in per cent of a mol and the amount of lactate in the same unit, and to compare the figures. When this comparison is made the amount of lactic acid is generally found to be too small to account molecule for molecule for the neutralization of $NaHCO_3$. But suppose the figures exactly balanced, would that show that there is a simple lactic acid acidosis after exercise? Many recent writers with the alkaline reserve in their minds have discussed this and related questions (e.g., ketosis) as if the logical answer were in the affirmative. In fact, however, if lactic acid or any other strong acid is added to blood, most of the alkali which neutralizes it is drawn

from the hemoglobin alkali reserve; and it causes, therefore, only a very slight decrease in the plasma bicarbonates. As Haggard and I pointed out, measurement of the "acid load of the corpuscles" (33) would be a much truer index of the decrease of the real alkaline reserve of the blood than the usual determination of plasma bicarbonates. Bock, Field and Adair (34) have recognized and applied essentially the same idea and find the diminution of bicarbonates is not a valid index of the amount of acid in the blood. Thus even on strictly chemical grounds it is erroneous to conclude that a reduction of 10 or 20 per cent in the blood alkali (combined CO_2) is due to lactic or any other acid, unless many times as much of that acid in neutralized form can be found in the blood and urine as would correspond to the reduction of alkali on a molecule for molecule basis. The same relations and the same reasoning apply also to the decrease of bicarbonates in ketosis.

In all such cases the "reductio ad ignorantiam" lead to the question: If the decrease of blood alkali is not due to neutralization by acid, to what else can it be due? No answer has been forthcoming, so lactic acid acidosis has been considered to be proved. In fact, however, the comparison of the amount of lactate and the amount of decrease of blood alkali (or combined CO_2) is sufficient evidence to prove that whatever the "acidosis" really is, it certainly is not due merely to a flooding of the blood and neutralization of its $NaHCO_3$ by lactic acid. Another possible and more probable direction in which an explanation may be sought will be suggested hereafter.

Defects of the capnial theory. The general conception of the regulation of the acid-base equilibrium of the blood which has been set forth in this review may be called the capnial theory (35), in contrast to the acid poisoning theory. The latter will probably be either discarded or greatly modified, and indeed it is constantly being modified in respect, for example, to the real alkaline reserve. But it must be freely admitted that the capnial theory is also still very defective in important particulars, and that some conditions predicted from it have been proved by competent investigators to have quite different values from the predictions. When this theory was formulated, essentially as here stated, some years ago by Haggard and myself (25), we believed that it afforded a nearly complete explanation of all the acid-base adjustments and readjustments in the blood. It appears to accord quite well with the conceptions regarding adjustment to altitude now used by all workers on that problem (11), (12), (14), (20), (24), (36). But we believed also that it explained the disturbance of equilibrium

and the readjustments occurring in anesthesia. From the theory, as expressed in the CO_2 diagram, it appeared to us that during the stage of respiratory excitement the pH and the A point on the dissociation curve should be forced over to the alkaline side. This would drive alkali out of the blood and lower the dissociation curve. During recovery the pH and A point would move to the acid side of normal, because of the depression of breathing, and alkali would thus be called back into the blood. The pH and A point in the CO_2 diagram would thus revolve counterclockwise in a roughly circular path and would control the passage of alkali out of the blood and into it again. In fact, however, several investigators (37) have shown that in anesthesia the pH or A point is continually on the acid side of the normal pH. Evidently the influence of anesthesia in respect to disturbance of pH is exerted, not only through the respiratory center, but also through some influence upon the tissues (probably upon the oxidation processes) causing them to draw alkali out of the blood, or to discharge phosphoric acid into the blood, as Stehle and Bourne (38) find.

This matter might seem to be one of purely theoretical interest. In fact, however, it is of so much practical importance that a large number of lives and several years of progress have been lost because of the difficulties which the current conception of "acidosis" has opposed to an adequate practical trial of therapeutic measures based on the capnial theory and in accord with the experiments on which it in turn is based.

Experimentally, Haggard and I (21) found that inhalation of CO_2 in slightly more than the alveolar concentration was a very effective method of restoring a low blood alkali to normal after anesthesia, or "calling alkali back into the blood," as we expressed it. We tried this procedure at first very cautiously, then more and more confidently on patients after prolonged surgical operations and profound ether anesthesia administered by the drop method (39). The clinical effects of this use of CO_2 inhalation were brilliant, a result confirmed by White (40).

We then tried with success inhalations of CO_2 and oxygen, or even CO_2 in air, for the treatment of carbon monoxide asphyxia. Carbon monoxide asphyxia is par excellence the classic form of "asphyxial acidosis." In it, if in any condition, should be found that "self poisoning by acids from incomplete oxidation in the tissues," which the current theory of acidosis postulates. Nevertheless our experience has demonstrated that in the treatment of carbon monoxide asphyxia, if one had to choose between pure oxygen without CO_2, and CO_2 in air with no

addition of oxygen, the latter treatment would be much the more effective even in overcoming acidosis (41), (42).

With these results established both experimentally, and now on an extensive scale clinically, in respect both to anesthesia and asphyxia, it might be supposed that it would have been easy to bring about the introduction into practical therapy of CO_2 inhalation. But it happened on innumerable occasions throughout many years that, just as success seemed to be at hand, we were stopped because the clinician with whom we were coöperating was informed by some biochemist that: "During and after anesthesia, and particularly in asphyxia, there is a condition of acidosis. The alkaline reserve and pH are subnormal, indicating an excess of acids. Obviously, therefore, to administer CO_2 and thus increase the acidity of the blood still further is unjustified and in fact distinctly and strongly contraindicated." Truly a little knowledge of physical chemistry is a dangerous thing in the interpretation of vital processes.

The use of CO_2 inhalation in connection with anesthesia, especially after ether, has now proved in practice so advantageous that anesthetists are very generally and increasingly using it. Furthermore the inhalation of oxygen plus 5 per cent CO_2 for the treatment of carbon monoxide asphyxia, has come into wide use (42). It has to its credit already the saving of a large number of lives. Of course the oxygen is responsible for much of the benefit of this inhalation and the CO_2 serves as a respiratory stimulant. But experiments on animals show conclusively that among the effects of CO_2 inhalation are restoration of a normal blood alkali, and probably of other interior conditions, even apart from the effects on respiration. Results on patients support this view. A workman is lifted out of a trench in the street or dragged away from a blast furnace; he is wholly comatose from carbon monoxide and scarcely breathing. He is given an inhalation of oxygen and 5 per cent CO_2 for perhaps a quarter of an hour after which he is not only conscious again, but ready to go back to work, feeling well and actively exerting himself. Longer asphyxia involves slower recovery, but the results are equally efficacious. Official reports of such cases, and sometimes of many such cases, come to us nearly every week. They indicate that the inhalation of oxygen and CO_2 does something more than merely to displace carbon monoxide from the blood. In experiments on men gassed up to 30 or 40 per cent saturation in this laboratory, no one who has not received CO_2 has ever felt like going back to work the same day. The early stages of carbon monoxide asphyxia

always involve much overbreathing. We originally thought that the resulting simple acapnia was the direct cause of the lowered blood alkali. It must be admitted now, however, that it is more probable that the withdrawal of blood alkali into the tissues is the result of tissue asphyxia. One result noted here is cerebral edema (43). Another is pulmonary congestion. Both are counteracted by oxygen and CO_2 inhalation. Whatever the explanation may be, the decrease of CO_2 in the body, decrease of both carbonic acid and bicarbonates, appears to be an important element in carbon monoxide asphyxia; and the restoration of this carbon dioxide to the body is an important element in effective therapy both after asphyxia and anesthesia.

SOME IMPORTANT, BUT NEGLECTED PHENOMENA. The problems discussed in this review require for their solution, not only the physical chemistry of the blood, but a much more extensive consideration of respiratory behavior than is usually brought to bear upon them. Many of the respiratory reactions are indeed difficult to explain, or at present even to harmonize with each other. But that does not justify ignoring them. Some of the phenomena, which are especially enigmatic or paradoxical are as follows:

A. If a normal man inhales a few breaths of nearly pure nitrogen a vigorous hyperpnea immediately results. It is a well-established fact that there is an interaction of oxygen and CO_2 upon hemoglobin, by which the removal of oxygen enables the blood to carry CO_2 more easily, that is, at a higher pH. The blood is therefore presumably suddenly rendered very much more alkaline than normal by the nearly complete removal of oxygen. Nevertheless hyperpnea is induced instead of apnea, as might be expected on the analogy of the effects of voluntary forced breathing. Evidently low oxygen does not act on respiration directly through pH.

B. If a normal man breathes air containing even 2 or 3 per cent less oxygen than normal (18 per cent instead of 21), his respiration usually exhibits after a time an increase of the volume of air per unit of CO_2 exhaled. If we compare the pressure of oxygen in the lungs in this condition and the pressure when normal air is breathed, and apply these pressures on the oxyhemoglobin dissociation curve, the difference in the indicated oxygen content of the arterial blood is very slight. It would seem logical, therefore, to infer that the stimulation induced by slight oxygen deficiency is due to decrease in the pressure of this gas dissolved in the blood rather than to a decrease in the amount (in volumes per cent) held by the blood.

C. Contrary to the view just stated, however, if the man is made to breathe air which is normal except for the addition of 0.01 to 0.04 per cent carbon monoxide, so that his blood after a time is 10 or 15 per cent saturated with this gas, the volume of breathing is also distinctly augmented per unit of CO_2 exhaled. In this case, however, the oxygen pressure in his blood is normal, or is even increased above normal by the slight hyperpnea. At the same time the oxygen carrying capacity of the blood is so slightly decreased that it seems impossible that the venous blood from any tissue can actually be depleted of oxygen, and

yet the effect on respiration is essentially the same as that of a slightly decreased pressure (as in *B*).

D. The breathing of low oxygen, or low atmospheric pressure, or carbon monoxide asphyxia do not decrease the amount of oxygen consumed even when the stage of coma is reached. On the contrary the oxygen consumption continues at almost the basic rate, or is even slightly increased by the muscular activity of breathing, until the degree of saturation of the blood is such that the venous blood is entirely depleted of oxygen and death is at hand. The idea that the effects on breathing and on blood alkali induced by the conditions above noted (*B* and *C*) are due to an absolute deficiency of oxygen is, therefore, very improbable (45).

E. When a dog is kept in a chamber containing air to which 0.1 or 0.2 per cent carbon monoxide has been added, it develops the initial symptoms of asphyxia, especially hyperpnea, and becomes groggy. If at that stage it is removed to entirely fresh air it is liable to collapse. City firemen and the men on mine rescue crews are well acquainted with this effect of fresh air on a man who has been working in smoke. Until recently no one had even suggested an explanation of these phenomena. Haldane (44) has now suggested that it is the CO_2 in smoke which keeps the men up, and that it is the lack of CO_2 which is the final element in the collapse in fresh air.

F. When a dog is kept in air to which enough carbon monoxide has been added to produce a very considerable, but not fatal, degree of saturation of its blood, it often happens that the animal collapses; but after a time a considerable degree of recovery may occur. The animal may not only recover consciousness, but may even walk about the gassing chamber in which a short time previously in the same atmosphere it had lain in a state of coma. It is upon such phenomena as these (although not quoting this particular occurrence) that Haldane (24) bases his belief in an active secretion of oxygen by the lungs as a factor in acclimatization to extremely high altitudes and to oxygen deficiency in general. Perhaps the accumulation of CO_2 in the chamber is also a factor.

G. When muscles are worked intensely the effect upon respiration is much greater than the gaseous exchange warrants (as Zuntz (46) showed), and distinct overbreathing may be induced. This phenomenon is most striking at low oxygen pressures or after partial saturation of the blood with carbon monoxide. Men out of training overbreathe much more than do athletes for an equal exertion (47). The shortness of breath of cardiac patients is similar. (Overworked muscles seem to produce respiratory X; but this statement merely denies that the overstimulation of respiration is due to CO_2, or in the main to lactic acid. According to Haldane's view, the overworked muscles produce also the something which causes active secretion of oxygen when the blood from these muscles reaches the lungs. Whether or not the two hypothetical explanations here mentioned are correct, the phenomena are real and require not denial but study and explanation.)

H. When both vagi are divided (in a dog under local but no general anesthesia) it is found that inhalation of CO_2 still produces almost as distinct augmentation of the volume of breathing as was the case prior to the section. In the experience of this laboratory, however, the breathing of air low in oxygen has

little and sometimes no perceptible effect on respiration after vagus section. The same is true of the induction of carbon monoxide asphyxia; although both of these conditions cause hyperpnea in the intact animal. The respiratory rhythm and volume after vagus section may continue almost unaffected up to the point of fatal asphyxia. The blood alkali in these cases usually continues to be normal up to the end (45). This is crucial evidence against the present general conception of the relation of acidosis to oxygen deficiency.

Such phenomena as these require to be considered, explained and harmonized before the behavior of the hemato-respiratory functions in health and disease can be regarded as solved. Such problems certainly are not solved by merely referring them wholesale to disturbance of pH. Nor are they solved by transferring them to the respiratory center itself. (Gesell, this volume.)

OXIDATION OF SUGAR IN THE TISSUES AS A CONTROLLING FACTOR IN THE REGULATION OF BLOOD ALKALI. The deficiencies above shown in both of the theories, in respect to the part played by the tissues in the causation of low blood alkali, indicate that the time is ripe for the formulation of a conception on that point more nearly adjusted to the present state of information. Such a formulation, on the basis of present data, will necessarily leave many of the physiological reactions above mentioned still unexplained; but it will assist in the attainment of new knowledge by serving as a *corpus vile* on which investigators may operate to show wherein and to what extent it is wrong. For this purpose the following propositions may serve: When the blood alkali is decreased in such conditions as diabetic acidosis, or as a result of carbon monoxide asphyxia, or general anesthesia (e.g., with ether) or after vigorous muscular exercise, the chief process involved is the withdrawal of alkali from the blood into the tissues; for the amount of (neutralized) acid found in the blood is usually slight in comparison to the decrease of the alkali of the corpuscles and plasma bicarbonate. The condition chiefly determining the extent of this withdrawal of alkali, or its return to the blood, is the freedom with which sugar is oxidized in the tissues. Among the essential factors are an adequate supply of sugar, an ample supply of oxygen, and sufficient insulin to facilitate the process. Deficiency in any of these factors leads to withdrawal of alkali from the blood into the tissues. In the dynamic equilibrium of this process the mass action of oxygen is balanced against the mass action of bicarbonate ions, and the H-ion concentration is a variable dependent (through respiration) upon the ratio of oxygen to bicarbonate and tending to restore that ratio to normal by the shift of alkali from blood to tissues and vice versa.

The form of buffering occurring in the tissues, for example in muscle,

is not so well understood as that in blood, but it appears to consist largely of proteins serving as weak acids and combined with inorganic alkalies. The condition would thus be analogous to the hemoglobin-alkali which is the chief factor in the buffering of the blood. It would exceed the scope of this review to discuss this topic extensively but some recent investigations which seem to be in accord with the formulation presented in the previous paragraph may be mentioned.

Benedict and Talbot (48) have shown that in children the reserve of sugar is much more rapidly exhausted than in adults; and Talbot, Shaw and Moriarty (49) find that hypoglycemia due to fasting produces a definite acidosis which quickly clears up on administration of even a small amount of sugar. They conclude that clinically when a ketosis is found it must be assumed that there is some interference either with the availability of the sugar or with the ability of the body to oxidize sugar. If the body is able to oxidize sugar, as is the condition in fasting children, then the ketosis must be due either to a sugar deficit or a lowered availability of the sugar deposits of the body. Clinically, the administration of glucose to a child with acidosis is usually all that is necessary to correct the symptoms of this condition if the supply of water also is ample.

The clinical experience and experimental work of Banting and his associates (50) appear to demonstrate that insulin promotes the combustion of glucose. Bock, Field and Adair (34) find that even in diabetic coma the administration of insulin and sugar may be largely effective in overcoming the clinical symptoms and restoring a normal blood alkali. It appears from the observations of John (51) among others that insulin does not act in the blood; presumably its action is in the tissues.

Allen (52) reports observations on puppies closely similar to those above noted in children. Acidosis is induced by sugar depletion and relieved by sugar administration. He shows both by experimental evidence and from clinical facts that low blood alkali is merely a concomitant and not the essential factor in acidosis as a clinical entity; and he urges a broader view than merely that the condition consists in a neutralization of the body's store of alkali by acids.

The work of Shaffer (53) dealing with the ketogenic-antiketogenic balance indicates a line along which much valuable information is accumulating. Allen justly remarks, however, of some of the too rigid conceptions based on Shaffer's work that it is necessary to consider not merely the two known chemical factors, glucose versus fatty acids,

but also a third variable, the living organism which does not necessarily or invariably deal with the same food mixtures in the same way under all normal and pathological conditions.

In all of these investigations we have evidence that the symptoms of acidosis are due either to the pharmacological action of the products of disturbed oxidation, or to a deep-seated metabolic disturbance, rather than to the mere neutralization of the body's alkali by acids. In large part the substances occurring are not acids but ketones and related substances. On this point a very significant observation is mentioned by Talbot and his associates (49) as made by Parsons. He found that 20 per cent acetone injected into the rabbit in doses of from 1.5 to 4 cc. depending on the weight of the animal was sufficient to cause shock and often death. If 5 cc. of 20 per cent glucose were given intravenously it was generally sufficient to bring the rabbit out of shock almost immediately and to cause all signs of acetone intoxication to disappear. Somewhat similar observations on post-operative shock in patients are reported by Fisher and Snell (54).

Evidently the utilization of glucose in the tissues is a factor on a par with respiration and with the activity of the kidneys. These three functions interact in the physiological regulation of the acid-base balance of the blood.

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INHIBITION

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Irritability is regarded as perhaps the most universal property of living matter and it is defined usually as the ability of a tissue to respond with its characteristic functional activity upon the application of an adequate stimulus. This definition seems to identify irritability with excitability, but, as Meltzer and others have pointed out, inhibition or the suspension of functional activity is also a characteristic of living matter that is widespread and plays in many instances an equally important rôle in the adjustment of the organism to changes in its external or internal environment. Meltzer (1) held extreme views as to the universality of inhibition. He considered that "each and every state of life . . . . is a resultant of the combination of the two antagonistic factors, excitation and inhibition." A muscle at rest, for example, is in state of inhibition. While views of this kind have not met with general acceptance it is to be noted that much the same conception is implied in Pavlov's (2) theories concerning the nature of the activity of the cerebral cortex. He pictures the cortex as made up of an infinitude of excited or inhibited points, each more or less rapidly changing its condition from one state to the other. Every point is either excited or inhibited, none apparently is at any time in a state of rest.

In his explanations of the mode of action of the cortical tissue Pavlov makes constant use of the property of internal inhibition, which is defined as an inhibition that develops gradually or is worked out under a given set of conditions. His general thesis is that when a conditioned reflex does not correspond with reality it is inhibited. Thus, to take a simple example, if a stimulus is applied to the skin and, at the same time, food is given a conditioned reflex is soon established, so that the stimulus to the skin, when used alone, will give the food reflexes (saliva, movements, etc.). When this conditioned reflex is first established it can be obtained also from neighboring regions of the skin, it is not localized to one point. A narrow locali-

zation of the reflex can be established, however, if the stimulation of one point is always accompanied by food while that of other points is not. The food reflex from the other points gradually diminishes and disappears, due to the development or working out of a process of internal inhibition, which in cases of this kind is classified as a differential inhibition. In some of the cases he describes it is difficult to understand why the process of inhibition is called upon to explain the result obtained. Thus, a conditioned reflex for food reactions may be established for a given musical tone, so that eventually the sound alone causes a secretion of saliva and the other accompaniments of feeding. If now the stimulus be repeated every two to three minutes its effect diminishes and the reflex finally disappears. It is extinguished by a process of inhibition. But an inhibition like an excitation presupposes a special stimulus and one asks in this case what is the stimulus that arouses the process of inhibition. What seems to be applied is simply a succession of excitatory stimuli unless one assumes that the animal's growing consciousness of the lack of reality supplies the occasion for the inhibition.

The great volume of important results obtained by Pavlov and his co-workers during a series of years offers insuperable difficulties at present to any satisfactory explanation in terms of the known anatomy and physiology of the cortex. As he says himself "es bleibt alles dunkel." This is particularly true for the phenomenon of internal inhibition. This form of inhibition may eventually prove to be identical in mechanism with the inhibition with which we are acquainted in the other tissues of the body, but at present they can not be compared. The internal inhibitions, like the conditioned reflexes, are acquired or worked out or conditioned. The variety of inhibition with which we are more familiar, and which by way of distinction is designated external inhibition, ensues practically at once upon the application of the stimulus, it is not worked out gradually. It is an inherited or unconditioned reaction. The example that we hold in mind is the case that was first discovered, namely, the inhibition of the heart beat upon stimulation of the vagus fibers. This review deals only with external inhibition.

Recognizing the existence of a state of inhibition one inquires naturally whether there are any properties or peculiarities characterizing this state as compared with that of rest or of excitation. Bayliss (3) in his interesting discussion of the subject defines inhibition as "any process which stops an action in progress by application of some in-

fluence from without," excluding from this definition of course such influences as cause an actual injury or destruction of the tissue. For it must be kept in mind that inhibition is a normal process that is in no way injurious to the cells. On the contrary, there is some evidence or at any rate some reason for believing that it is beneficial in the sense that it increases the power of the cell to do work. Bayliss further suggests that inhibition may act in one of two general ways. It may quell a state of excitation or it may act by interposing a block or resistance that the excitation can not overcome. By either method the state of inhibition produced would seem to be identical with that of rest or inactivity, or, as Gaskell expressed it, the effect is neutral.

Little is known about the actual conditions in the inhibited tissue, the nature of the metabolism, for example. A few observers have studied the respiratory process in the inhibited heart. Barcroft and Dixon (4) report that in the partially inhibited cat's heart there is a diminution in the oxygen consumption and carbon dioxid elimination.

	GAS PER GRAM PER MINUTE	
	O_2	CO_2
Before stimulation	0.014	0.038
During stimulation	0.009	0.005
After stimulation	0.022	0.015

Wolfsohn and Ketron (5) obtained a similar result upon the totally inhibited heart of the dog. Their figures are given in percentages of the blood of the coronary vein.

	O_2	CO_2
Before stimulation	0.112	0.362
During stimulation	0.118	0.327

Barcroft (6) quotes his own results and the rather incomplete data given by Rhode (7) as evidence against Gaskell's anabolic theory of inhibition. He argues that on this theory there should be a storage of oxygen during inhibition which seems from the above figures not to be the case. In view of the incompleteness of the data this does not appear to be a justifiable conclusion. The oxygen taken up by the heart seems to have been much greater than in the case of resting

muscle. Chauveau and Kaufmann (8) for the horse and Verzar (9) for the cat obtained a figure of 0.003 cc. per gram of muscle per minute as contrasted with 0.009 cc. in the experiments of Barcroft and Dixon for the inhibited heart. But in this case the heart was only partially inhibited. It would be interesting to know the oxygen consumption in the fully inhibited heart with an artificial circulation under constant pressure. Experiments of this kind might be made for short periods on the mammalian heart and for long periods on the heart of the cold-blooded animals. The most positive results bearing upon this point were obtained by Garrey (10) from experiments made upon the motor ganglia of the heart of the Limulus. These ganglia lie upon the surface of the heart and may be removed together with the inhibitory nerve running to them. The author had demonstrated the rate of production of CO_2 in these nerve cells by immersing them in a solution containing phenolsulfonephthalein and noting the time necessary to obtain a change in reaction from pH 7.8 to pH 7.4. Using the same technique he found that during the stimulation of the vagus nerve the production of CO_2 was reduced from 20 to 60 per cent. Inhibition, therefore, diminishes the chemical reactions in nerve cells that lead to the production of CO_2. On cessation of inhibition the rate of formation of CO_2 returned gradually to normal but there was no increase beyond normal. This latter fact is cited by Garrey as an argument against Gaskell's anabolic theory of inhibition.

Gaskell (11) has noted one apparent difference between the condition of the inhibited heart-muscle and that of the resting muscle, namely, the slightly greater positive potential exhibited by the former. Gaskell's well-known results upon the terrapin's heart have been corroborated by Meek and Eyster (12), by Samojloff (13) and by Garrey (14). Einthoven (15) believes that this result is an artifact due to the increased state of mechanical tension to which the heart is exposed during vagus stimulation owing to the slow sinking of pericardium and heart caused by the contraction of the lungs. Garrey's experiments, however, seem to meet this criticism. The effect was obtained, for instance, on the excised isolated auricle of the alligator's heart and was eliminated by atropin applied locally to the heart tissue.

A further difference in condition between the inhibited and resting state is furnished possibly by the loss in irritability or responsiveness caused by inhibition. Biedermann (16) states that the adductor muscle of the forceps of the crab when inhibited through stimulation of its nerve supply bcomes less excitable toward artificial stimuli.

McWilliam (17) showed that the heart tissue in the newt and eel loses its excitability under vagus stimulation and Dale and Mines (18) found that conductivity was diminished from vagus stimulation. So far as the heart tissue is concerned, these results are made doubtful by the recent work of Ishikara (19). This observer studied the curve of recovery of excitability in the refractory period in the different chambers of the heart of the toad and found that the height and form of the curve are not influenced by vagus inhibition. He concludes that inhibition does not affect either the excitability or conductivity of the heart tissue. When the auricle is brought to rest by vagus inhibition, a stimulus applied to it may excite no contraction of the auricle, but it does arouse an excitation impulse which is conducted to and causes a contraction of the ventricle. His interpretation of the effect of the inhibition is that it represses the formation of the inner (autochthonous) stimulus, to which must be added also apparently a depression of contractility as distinguished from excitability. In some way therefore the conditions within the heart tissue are stabilized during inhibition as compared with the state of rest.

Many observers have compared the inhibitory state with the refractory state. Verworn in fact sees in inhibition simply a prolonged refractory state but it is to be noted that if Ishikara's results are correct the resemblance between inhibition and the refractory state is superficial only. In the absolute refractory period there is a total loss of excitability with a gradual recovery during the relative period, whereas in the inhibitory state excitability is normal and there is production of and recovery from a refractory state after excitation.

Verworn (20) has laid emphasis upon the fact that the state of inhibition is localized; it is not transmitted or conducted like the excitatory state. Whether this is true in all cases of inhibition would be difficult to say in view of the meagerness of our information. In Biedermann's (21) well-known experiments upon anodic and cathodic inhibition of the beating heart or of the veratrinized muscle the inhibition was localized to the polar region, there was apparently no propagation of the "excitatory inhibition," although the author states that the possibility of such a propagation is not to be denied. According to Verworn, inhibition of a spinal nerve cell through afferent paths causes no diminution in the excitability of the axis cylinder process of the cell. Pavlov on the contrary adopts a different view in regard to the state of internal inhibition in the cerebral cortex. He assumes that this spreads, like that of excitation, from its point of

origin over wide areas of the cortex, and he speaks of inhibitory impulses as being transmitted from point to point. Whatever may be the possibilities in the case it seems certain as regards external inhibition that we have no conclusive evidence of the conduction of the inhibitory state in any of the peripheral tissues in which inhibition is known to occur. There is no evidence to indicate that there is anything of the nature of a self-propagating inhibitory impulse or process comparable to the wave of excitation. In the light of the foregoing facts it would seem to be inadequate to define inhibition simply as the quelling or blocking of an action in progress. It is something more positive and characteristic, but a satisfactory definition must wait upon a better knowledge of what actually takes place in the inhibited cell.

Among the peripheral tissues we meet with the phenomenon of inhibition in rhythmic muscle, such as that of the blood-heart or the lymph-hearts, and in the tonically active plain muscle of the abdominal and pelvic viscera, the blood-vessels, the iris, etc., possibly also in the case of the glandular tissues. In the central nervous system the cells or centers that exhibit tonic activity, such as the respiratory and vasomotor centers, are clearly subject to inhibitory control through afferent paths, and it would seem probable from the study of spinal reflexes that in the reaction of one neurone upon another inhibition is almost as frequently in evidence as is excitation. The value of the process as an aid in coördination and adaptation is readily understood. In a rhythmically beating heart there is need for a means of slowing as well as of quickening the rate, and the value of the inhibitory regulation is indicated by the widespread occurrence of inhibitory nerves to the heart among the invertebrates as well as the vertebrates. So also in tissues exhibiting intrinsic tonus the necessities of adaptation call for both an increase and a decrease in the state of tonus. The case is different for the skeletal muscles of the vertebrates. There is no good evidence that they possess inhibitory nerves, or that influences from without can produce in them an inhibitory state other than the transitory phase of refractivity that follows excitation. The tonicity exhibited by this tissue is neurogenic, and the processes of adaptation and regulation are effected through the governing motor centers of the central nervous system. These centers are subject to reflex inhibition as well as reflex excitation but in the muscles themselves apparently only excitatory processes occur.

Theories of inhibition. Since the discovery of inhibition of the heart

in 1845 many theories have been proposed to explain the nature of the process. Some of these theories have been intended to account for the phenomenon in all tissues on the assumption that inhibition wherever it occurs is fundamentally the same process. Other theories have been proposed specifically to explain the process as it takes place in the central nervous system. Many authors have been inclined to the view that inhibition may be produced by different methods; that the process in the central nervous system, for example, may be quite different from that operating in cardiac inhibition. The natural increase in knowledge that has come from investigation has served to make a certain number of these theories improbable, but for the sake of completeness a brief reference may be made to the more prominent views that have been proposed.

The interference theory. von Cyon (22) (1870) utilized the analogy of the summation or interference of light or sound waves to suggest that two nerve impulses falling simultaneously into a nerve cell might be summated or obliterated according to the phase in which they met. The theory was adapted especially to explain the cases of inhibition in the central nervous system from the concurrence of two different afferent stimuli. From what we know of the character of the nerve impulse the theory has no claim for serious consideration, and it was subsequently abandoned by Cyon in favor of a theory of resistance. As Bayliss has pointed out in his review of the subject interference of wave motion implies a positive and a negative phase in the waves, interference occurring when the crest of one wave coincides with the trough of another. In the nerve impulse we have only one phase. The negative potential rises from a base line to a maximum and then falls again to zero. By analogy with sound or light waves only a summation would be possible from the concurrence of two impulses.

The assimilation or anabolic theory. E. Hering (23) first suggested the thought that the processes of synthesis or assimilation are responsible for a physiological state opposite to that accompanying the processes of breaking down or disassimilation. He applied this conception to his well-known theory of color vision and subsequently in a speculative way to many problems of physiology including that of inhibition. Disassimilation results in functional activity, contraction for example, while assimilation leads to inhibition. Gaskell (24) (1887) adopted and elaborated this view. He supposed that the heart and the vascular and visceral musculature in general receive two sets of nerve fibers; one, the excitatory fibers, arouse catabolic or

disintegrative changes, with liberation of energy, which result in contraction, the other, the anabolic fibers, cause anabolic or integrative changes which lead to relaxation or inhibition. On this view inhibition should be a beneficial process since it implies a storing up of potential energy. Gaskell gave some experimental evidence for his hypothesis, first, by attempting to show that the contractility of the heart-muscle is in fact improved by inhibition and, second, by his demonstration that the inhibited heart-muscle exhibits a positive potential, whereas the excited muscle shows a negative potential as compared with the resting tissue. But this evidence has not been accepted as satisfactory. If we grant that the inhibited muscle has a positive potential this fact in itself is no proof that anabolic changes are taking place. The usual connection between catabolic changes and the development of a negative potential does not justify the conclusion that anabolic changes must be accompanied by a positive potential. If this latter condition is due to a concentration of positively charged ions we can not infer that such a concentration must arise from synthetic changes. Theoretically it might as well or more likely result from catabolic processes. His other belief that inhibition improves the condition or working power of the tissue has not been confirmed generally. While it is referred to frequently in the literature in connection with inhibition of the heart it has not been satisfactorily corroborated.

The theory falls in with the general idea that the inhibitory changes must be of an opposite kind to those causing excitation but, as McDougall has pointed out, the hypothesis of an increased anabolism during inhibition is not essential to the theory. It would be simpler to assume that the effect of inhibition is to decrease the catabolic changes. Bayliss makes a similar objection when he states that it would be more reasonable to suppose that an increase in anabolism, if it occurred, would cause a parallel increase in catabolism, just as in a chemical system an increase in the amount of the reacting mass increases the total reaction taking place. The fundamental objection to the theory at present is that it is at variance with modern conceptions of the energetics of contraction and relaxation. It was built upon the old conception of the living protein as a hugely complex molecule which was continually undergoing disintegration in functional activity and was constantly being reconstituted or synthesized from the food material, as illustrated in the biogen hypothesis or the conceptions of Pflüger in regard to living protein and the storage of

oxygen. The belief at present is that the energy of contraction comes from the breaking down of carbohydrate material which is, so to speak, outside the organized or living tissue. The process of the breaking down and building up of glycogen does not fit into the anabolic theory of inhibition. The theory has not grown in favor in recent years and has not obtained any additional experimental support since Gaskell's work.

The drainage theory. McDougall (25) (1903) proposed a theory of inhibition in which the transmission of energy along a nerve fiber was compared with the flow of liquid through a conduit. If water is flowing in a series of pipes under a given head of pressure and the resistance is lowered in one of the pipes more liquid will flow along that path and less along the others; liquid is diverted from the latter channels or we may say that the flow in them is inhibited. He assumes, in terms of this analogy, that when a nerve is stimulated it becomes a path of lower resistance and deflects to itself some part of the energy that was flowing along other fibers, these latter, therefore, showing the phenomenon of inhibition. "Inhibition appears always as the negative or complementary result of a process of increased excitation in some other part." Specifically his hypothesis was devised to explain the fact of reciprocal innervation in which reflex excitation of one muscle, flexor or extensor, is accompanied by inhibition of the antagonist. He gives an anatomical schema for the innervation of the flexors and extensors which would work in this manner if his principle were correct, that is, stimulation of a flexor muscle would drain off tonic impulses flowing to the extensor, and vice versa. For other cases of inhibition, that of the heart, for example, the theory can not be applied. Outside the objection that the theory is entirely analogical and is not supported by anything of the nature of experimental evidence, it involves, as Bayliss has pointed out, a fundamental misconception of the nature of the nerve impulse. Nerve energy is not a definite limited quantity such that when part of its passes through one branch of a nerve less will be left for the remaining branches. Quite the contrary is true. When a nerve fiber branches a disturbance in the main stem is propagated with maximum energy in all the branches no matter how numerous. The energy liberated is in potential form in the fibers themselves and the better analogy is to a train of gunpowder rather than to a flow of water under a certain head of pressure.

The colloidal theory. Macdonald (26) has imagined a theory in

which excitation and inhibition are dependent upon the state of dispersion of the colloid material composing the irritable tissue. Excitatory stimuli produce a coagulative change or a state of aggregation in the colloid, which results in a liberation of the bound electrolytes, potassium salts for example, to the external phase. The unequal diffusion of the ions of these salts leads to a potential difference which is at the basis of both excitation and inhibition. The theory assumes that the negatively charged ions diffuse more rapidly and cause in the next succeeding region a coagulation change with liberation of more electrolytes and thus the disturbance is propagated. The positively charged ions on the contrary give rise to a more perfect dispersion which is followed by a more complete adsorption of electrolytes. Inhibition is defined as the result of the more complete dispersion and excitation as the result of the change toward increased aggregation. As Bayliss expresses the theory, an aggregation by diminishing the adsorptive surface increases the concentration of electrolytes in the external phase and thus gives rise to activity or stimulation. A greater dispersion of the colloid particles has the reverse effect, the adsorptive surface is increased and there is diminished activity or inhibition. At the time that it was proposed it seemed to some of the leading workers that this theory might contain the germ of a real explanation of the processes of excitation and inhibition, but in recent years no attempts have been made to elaborate it or to bring it into relation with experimental data. It is obvious that it can not be applied directly to the specific case of the heart in which stimulation of one nerve causes excitation and stimulation of another inhibition.

The synaptic membrane theory. In the course of his extensive and important investigations of reflex activities Sherrington (27) was led to believe that the seat of inhibition as well as of excitation lies in the contact surface or membrane at the synapse connecting two neurones. On this general view he concludes that inhibition is referable to a change in the synaptic membrane causing a block in conduction. The phraseology used would seem to imply that inhibition consists simply in a suppression of an excitatory impulse, but in other places this author expressly states his belief that inhibition does more than to stop an acting stimulus. For example, the effect of an exciting stimulus in some cases is to set up an after action or discharge in the neurone acted upon. In these cases inhibition through another path not only suspends the excitation but suppresses also the after-discharge. Sherrington's view says nothing in regard to the nature of the change responsible

for inhibition, it defines simply the locus of the action. In this latter respect his conclusion has been accepted quite generally and not only for the central nervous system but for the peripheral tissues as well. The "junctional tissue" has been regarded as a special mechanism through which the effect of the exciting nerve impulse upon the peripheral tissue is controlled.

In recent years two entirely different theories have been proposed to explain the phenomenon of inhibition. One of these theories is adapted mainly if not solely to account for inhibition as it occurs in the central nervous system; the other is meant to apply to peripheral inhibition or specifically to the inhibition of the heart.

The impairment and decrement theory. This theory was proposed by Lucas (28) and has been adopted and extended by Adrian (29). Inhibition is interpreted as an extinction of an excitatory impulse when the stimulation is rapid or strong owing to two facts, first, that the stimuli fall into the period of incomplete recovery, the relative refractory period, and therefore give rise to an impaired or less intense excitation, and second, that the junctional tissue or synapse is a region of decrement in which an impulse of diminished intensity will die out. The theory seems to have been devised to explain the paradoxical results obtained by Schiff and Wedensky from the stimulation of fatigued muscle-nerve preparations, and by Wedensky (30) in the stimulation of a nerve in which there was a narcotized area between the point of stimulation and the muscle. Under these latter circumstances Wedensky found that while a weak or slow stimulation might get through the narcotized area and cause a contraction, a stronger or more rapid stimulation was ineffective so far as the muscle was concerned. The explanation offered by Lucas is that the narcotized area is a region of conduction with a decrement as shown by the experiments of Adrian. When the rate of stimulation is increased the stimuli may fall in the relative refractory period at the time when irritability is below normal so that a reduced or impaired impulse is aroused which can not get through the region of decrement. The theory furnishes a satisfactory explanation of the Wedensky experiment and it is based upon experimental data.

Quite recently, however, Kato (31) has given reasons for doubting the hypothesis of conduction with decrement in a narcotized region. He finds that a nerve impulse in passing into a narcotized area suffers a diminution in intensity, but, if conducted at all, is conducted without decrement. This result as applied to Lucas's explanation of the

Wedensky experiment would mean that the reduced impulse was extinguished at once at the beginning of the narcotized area instead of disappearing gradually by decrement. According to this view an increase in rate of stimulation may change an excitation to an apparent inhibition by extinction of the impulse. An increase in strength of stimulus might have the same effect, since the stronger simuli would excite the nerve earlier in the period of recovery and thus give rise to a reduced or impaired impulse. As applied to the central nervous system under normal conditions the theory assumes that a synapse is a region of decrement and that, as in the case of the Wedensky experiment, an increase in rate, such as may happen from the simultaneous stimulation of two afferent paths, may bring about inhibition.

Lucas discusses his theory with great fairness and admits that there are certain experimental facts which it does not explain, such as the experiment by Sherrington in which it was shown that an excitatory stimulus may break through a simultaneous inhibition and cause a contraction. He also believes that "there may be several distinct forms of inhibition in the central nervous system, one depending on the extinction of impulses and another on a general depression of function in the inhibited tissue." It is important to determine if possible whether inhibition by extinction does really occur in the central nervous system. If it does then we may have inhibition without the presence of distinct inhibitory nerves. One and the same nerve unit when stimulated may give excitation or inhibition according to the rate or strength of the stimulus.

As regards inhibition in the peripheral tissues the facts known all seem to be opposed to the Lucas theory. Hunt's (32) experiments upon the simultaneous stimulation of the vagus and sympathetic fibers to the heart and Asher's (33) similar experiments with vaso-constrictor and vaso-dilator fibers to the salivary gland show that the resultant effect on the heart tissue is the algebraic summation of the separate effects and not an intensified inhibition. In the sciatic nerve Bowditch and Warren (34) have shown that rapid stimulation causes vaso-constriction (excitation) while slow stimuli give vaso-dilatation (inhibition), the reverse of what would be expected on Lucas' theory. In these and similar cases the explanation usually accepted is the existence of two independent sets of fibers, one causing only excitation, the other only inhibition. One set of experiments reported recently by Veach (35) would appear to support Lucas' theory of inhibition by extinction. This author describes the effect of changes in the

frequency and intensity of stimulation of the vagus nerve upon the contractions of the esophagus and stomach. It was found that low frequencies and intensities of stimulation cause pronounced contraction, while an increase in rate or strength of the stimulus, after an initial contraction, leads to relaxation as long as the stimulation continues. But upon the cessation of the stimulation there was a strong after-contraction, a result which seems to be entirely irreconcilable with an extinction theory. In this case also it seems probable that we are dealing with the simultaneous stimulation of separate motor and inhibitory fibers. Veach himself accepts his results as being of the same fundamental nature as the Wedensky inhibition. Without offering convincing reasons he rejects the alternative explanation of separate inhibitory fibers in favor of the view that rapid stimulation causes reduced impulses traveling in a relative refractory period of a conducting mechanism in the muscle itself. To explain the after contraction he is forced to construct subsidiary hypotheses in regard to the mode of action of the peripheral neurones.

In regard to the tonic centers in the central nervous system, for example, the respiratory, the vasomotor and the cardio-inhibitory centers, the evidence from experimental physiology supports the view that these centers are supplied with two different sets of afferent fibers, one giving only excitation, the other only inhibtion. When one is balanced against the other an algebraic effect is obtained as in Bayliss' (36) neat experiments with the depressor and anterior crural nerves in the rabbit.

For the phenomenon of reciprocal innervation with its simultaneous excitation and inhibition Lucas constructs a special schema, with two relays on each reflex path, which on his theory might account for the opposite effects of an afferent stimulus on the antagonistic muscles. But in this case he is obliged to make certain assumptions, both anatomical and physiological, for which experimental data are lacking. His explanation has the advantage that it calls only upon known properties of nerve fibers, but the more probable view would seem to be that the mechanism here is essentially similar to that known to exist in the peripheral tissues. This is essentially the position taken by Sherrington. He believes that the afferent fibers from the limbs divide in the cord into two branches, one causing excitation and one inhibition. A similar view is adopted by H. E. Hering (37) in his review of the subject. According to Hering the centripetal and intracentral fibers connected with muscular activity possess a double

function, which is to be explained on the theory that each fiber has a double ending (ein doppeltes Wirkungsende). Through one termination the muscle is set into activity, through the other the acting muscle is made to relax.

The main support of the theory proposed by Lucas comes from its application to the inhibition arising from the interference of two simultaneous afferent stimuli in the central nervous system. It is stated by many writers that inhibition may be produced by simultaneous stimulation of two spinal roots, although stimulation of either root alone causes excitation. If this statement is extended, as is usually done, by implication at least, to mean that the concurrence of two excitatory stimuli falling into the same nerve cell results in an interference or inhibition then it would seem, indeed, that there is a kind of inhibition in the central nervous system that is sui generis, and to explain the phenomenon some such theory as that advanced by Lucas must be accepted. Nothing of this sort is definitely known to occur in the peripheral tissues. The recent experiments of Cattell and Stiles (38) seem to demonstrate that when two excitatory stimuli fall into the same muscle fiber only summation results. Writers who claim that this peculiar kind of inhibition occurs in the spinal cord quote usually the experiments of Vészi (39) upon simultaneous stimulation of pairs of posterior roots in the frog, but a careful reading of the results of this author does not bear out the conclusions that have been drawn from them. Vészi exposed the 7th, 8th, 9th and 10th posterior roots and, after sectioning them, stimulated the central ends and recorded the effect upon the isolated gastrocnemius muscle. Contractions were obtained easily from the 9th root and also, although less readily, from the 10th. The 7th seemed to have practically no motor connections with this muscle. Since the 9th and 10th roots give motor reflexes with a final common path to the gastrocnemius the effects of a combined stimulation of these two roots are most significant. What he actually found was that when the strength of stimulus was sufficient to give a reflex contraction the combined stimulation of the two roots gave only summation. In order to inhibit the reflex contractions caused by stimulation of the 9th root it was necessary to stimulate the 10th root with stimuli so weak that taken alone they gave no motor effect. So also stimulation of the 7th root simultaneously with that of the 9th always caused inhibition of the motor reflexes from the latter, but in this case stimulation of the 7th alone had no visible effect on the gastrocnemius. These experiments therefore

do not seem to show that the simultaneous stimulation of two afferent paths, each of which is excitatory when taken alone, causes an inhibition. Whenever there was proved excitation from each root, their concurrence resulted in summation and not inhibition, as is the case in the peripheral tissues. Inhibition was obtained when the motor excitations from the 9th root were combined with non-motor excitations from the other roots, and it is quite possible therefore that such cases may be explained on the view that the motor cells innervating the gastrocnemius are supplied by separate afferent excitatory and afferent inhibitory paths, the latter reaching the cells mainly through posterior roots other than the 9th. This interpretation of Vészi's results is borne out by a consideration of the experiments reported by Baglioni (40) upon the motor and inhibitory effects of two simultaneous stimuli applied to the skin of the frog. He sums up his results in two general statements.

1. When the two stimulations are heterogeneous, one being a biologically useful and the other a nocuous stimulus, the latter prevails. It is misleading in this case to speak of the reflex due to the first stimulus as being inhibited. What happens is that it is displaced or overcome. The common group of muscles involved cannot execute two different combinations of movements at the same time and the stronger response from the nocuous stimulus prevails. 2. When the stimuli are homogeneous, both biologically useful or both nocuous, the effects are summated if the two stimuli are applied to the same reflex zone. If applied to different zones there is an antagonism or apparent inhibition which is in reality a compromise or algebraic summation, so far as the response of the effector muscles is concerned.

None of these cases force us to believe that one and the same path may give excitation or inhibition according to variations in the rate or strength of the stimulation, and until a reaction of this character is shown definitely to take place in the central nervous system there would seem to be no necessity to assume that the processes imagined by Lucas actually play an important rôle in the coördinating activity of this tissue. Certain simple cases of inhibition which seem to show the contrary are open at least to other explanations. Thus, in the decerebrate frog the croaking reflex aroused by cutaneous stimulation of the skin of the flanks may be inhibited by a strong cutaneous stimulus applied elsewhere. But in this case Schlösser (41) asserts that what happens as the result of the latter stimulus is not an inhibition of the reflex, but an antagonistic contraction of the adductors of the glottis.

Inhibition as a prolonged refractory period. Verworn (42) who had originally accepted the principle of the Hering-Gaskell theory later abandoned it in consequence of the experiments done in his own laboratory and elsewhere which seemed to show that in the central nervous system two excitatory stimuli of a certain rate or strength may so react as to cause an inhibition. He assumes that inhibition is identical with the refractory state and that under certain conditions of stimulation a refractory state or a relative refractory state may persist during the stimulation. He summarizes his views in the statement that "The processes of inhibition are simply and solely an expression of a refractory period persisting as a result of dissimilatory exciting stimuli." He makes the unproved assumption that excitation is due to an oxidative decomposition in the fiber, and that in a series of excitations following at a certain rate there is first a diminution of irritability owing to fatigue, that is, a relative deficiency of oxygen, and finally an equilibrium between ineffective excitations and recovery processes which keeps the fiber in a relative refractory state, so that no visible response is obtained as long as the stimulation lasts. The theory is based upon a conception of the nature of the excitatory process in the nerve for which there is no conclusive experimental evidence. The rôle of oxygen in the functional activity of the nerve is not known, but analogy with the better known processes in the muscle would indicate that it is probably concerned in the recovery from rather than in the production of excitation. In general this theory resembles that of Lucas in that it attempts to explain central inhibition as a special case by reference to known properties of the nerve fiber which vary with the manner of stimulation. But unless it can be shown more satisfactorily than has been done, that inhibition in the central nervous system is really a special case different from the phenomenon as we meet it in the peripheral tissues there would seem to be no occasion for such theories. The matter all seems to turn upon the point as to whether inhibitory paths are separate from excitatory paths, or whether one and the same path may give both excitation and inhibition according to the conditions of stimulation.

Humoral or chemical theories of inhibition. The somewhat inadequate term humoral may be used to designate some recent theories of inhibition which are applicable in the first place to peripheral inhibition and which are based upon the belief that the inhibitory nerve fibers cause the production of specific chemical products in the peripheral tissues. The inhibitory nerves inhibit by the production of

an inhibitory substance. Influenced by the similarity between the inhibition produced in heart tissues by excess of potassium salts and by stimulation of the vagus, Howell (43) proposed the theory that the effect of vagus stimulation is to cause an increase in the diffusible potassium in the tissue. The impulses brought to the heart through the vagus were supposed to cause the breaking down of some organic compound with the liberation of potassium in diffusible form, the action taking place especially at the venous end of the heart where the vagus fibers terminate and affecting first of all the site of the normal origin of the beat. The theory was supported experimentally by chemical analysis of the circulating liquid in an isolated mammalian heart during periods of inhibition as compared with periods of normal rhythmic contractions. The results seemed to show that the effect of prolonged inhibition is to cause a perceptible increase in the concentration of potassium in the limited quantity of circulating liquid supplied to the heart. No independent confirmation of these results has been published, but Loewi (44) has described some striking experiments which lead to a similar conclusion in regard to the mechanism of cardiac inhibition. He finds that when the heart of the frog or turtle is inhibited by stimulation of the vagus something is given off to the circulating liquid which has the power to cause inhibition in another unstimulated heart when circulated through it. Stimulation of the vagus nerve seems to cause the production and liberation of an inhibitory substance. Since the action of this inhibitory substance is suspended by the use of atropin, which is not the case in potassium inhibition, Loewi concludes that it is an organic substance possibly of the nature of neurine. Loewi's results have been extended by some ingenious experiments reported by Brinkman and Van Dam (45). Making use of two frogs they arranged an artificial circulation in such a way that the heart of one frog and the stomach of the other were in the same circuit, that is, the circulating liquid after passing through the heart of one frog was conveyed by a connecting tube to the vessels of the stomach of the second frog. They found that when the vago-sympathetic nerve to the heart was stimulated in the first frog something was given off to the circulating liquid which affected the stomach of the second frog. If an inhibitory effect was obtained upon the heart then the circulating fluid caused contraction of the stomach musculature. If an accelerator effect was shown by the heart then the liquid caused relaxation or inhibition of the muscle of the stomach. These results confirm those of Loewi in showing that stimulation of

the cardiac nerves causes the production of certain chemical substances which are given off to the circulating liquid, but they do not prove the formation of definite inhibitory or excitatory substances. The vagus fibers are inhibitory to the heart and excitatory to the stomach; with the sympathetic fibers the relations are reversed, and what the experiments tend to show is that stimulation of parasympathetic fibers to the heart leads to the production of chemical substances capable of stimulating the endings of parasympathetic fibers in the other tissues and the same for the sympathetic fibers. The illustrations given in the paper are not very convincing and it would be desirable to have the results confirmed by other observers. There is some experimental evidence, therefore, that stimulation of inhibitory nerves may cause the production of a specific substance through which inhibition is brought about. While the evidence is not conclusive it is suggestive and if confirmed by further work will help to explain why an excitatory impulse travelling down an inhibitory nerve gives a different end reaction from that caused by a similar impulse passing along a motor nerve.

DISCUSSION

The several theories of inhibition outlined above have little common ground that might serve as a basis for a general discussion. For the most part they are fundamentally different one from the other. But the experimental data collected by various workers, while oftentimes difficult to interpret and sometimes contradictory, suggest certain tentative deductions in regard to the physiological mechanism of inhibition, which may be stated briefly as follows.

1. Inhibition is fundamentally the same process in all tissues, and it is probable that a general theory of inhibition may be formulated to cover all cases. The writers who have felt otherwise have been influenced mainly by the character of the phenomenon as exhibited in the central nervous system, but the evidence for a special kind of inhibition in this tissue outside the internal inhibition attributed to the cortex, is not so convincing as to exclude the alternative view that wherever inhibition is encountered the underlying process and mechanism is the same.

2. Inhibition is produced in all cases through specific inhibitory nerves. So far as peripheral inhibition is concerned the evidence for this conclusion is strong and perhaps generally accepted. This assumption is made in regard to inhibition of the heart, the blood vessels,

the plain muscle of the abdominal and pelvic viscera, the musculature of the crustacean claw, the shell of the bivalves, etc. The same hypothesis holds also for afferent inhibition of the tonic centers of the central nervous system, such as the respiratory, the vasomotor and the cardiac centers. Reflex excitation and inhibition of these centers seem to be effected through separate and distinct fibers. For the general coördinating centers we are at liberty to assume such an arrangement as that suggested by Sherrington for the case of reciprocal innervation, that is, that the motor neurones have certain afferent connections which are definitely inhibitory, under normal conditions, and others that are definitely excitatory and that true inhibition is dependent upon a fixed mechanism which when stimulated gives always the same response. The arrangement may be of the same character as that shown by Carlson (46) to exist for the nerve ganglion of the heart of Limulus. This author has given very strong evidence for the view that the inhibitory fibers to the heart in this animal end in and affect the motor nerve cells that normally control the rhythmic beat. The inhibition is an inhibition of nerve cells, and the accelerator fibers which are also present terminate in all probability in the same cells. We must assume in this case that the nerve cells possess separate afferent connections with inhibitory and excitatory fibers.

3. Inhibition is determined by the nature of the ending. If one grants the general principle of separate excitatory and inhibitory paths or fibers it would seem necessary to conclude either that the nerve impulses conveyed along these paths are different and that there is such a thing as an inhibitory as well as an excitatory impulse, or else that the paths terminate in different mechanisms in the cells. In regard to the first of these alternatives the evidence from experimental physiology is altogether against it. When a motor fiber is forced to grow along an inhibitory path it causes inhibition when stimulated, and vice versa. Results of this character have been published by Langley (47) for the vasomotor nerves and by Erlanger (48) for the cardiac nerves. It may be supposed that the inhibitory fibers end in a specific physical or chemical mechanism whose reaction to excitation is different from that of the mechanism in which a motor fiber ends.

4. The reaction of the terminal mechanism may be chemical or physico-chemical. The difference between an inhibitory termination and an excitatory termination may be due to some difference in the physical structure or properties of the junctional tissue, the synaptic

membrane, or on the other hand there may be specific receptive substances each reacting to stimulation by the formation of a chemical product that in one case excites to functional activity and in the other brings about a state of inhibition. In the case of the heart some evidence has been furnished for the latter point of view, and theoretically, at least, a similar arrangement might exist in other peripheral tissues, and also in the central nervous system, for it is possible that in any nerve cell certain of the synapses may be excitatory and others inhibitory.

These generalizations do not touch upon the nature of the inhibitory process or reaction. There is almost no information on this point nor indeed is there anything except speculation on the more evident phenomenon of excitation. Presumably a knowledge of one process will throw light upon the nature of the other. What is sugested is simply that the anatomical-physiological mechanism of inhibition as exerted through the agency of the nervous system is similar throughout and acts upon chemical systems in the cells. Excitation and inhibition may be effected not only through nerve impulses, but by chemical messengers or hormones. Schafer (49) has emphasized this point. He proposes to limit the term hormone, in accordance with its etymology, to those members of the group that excite functional activity and to designate as chalones those that suppress activity or cause inhibition. It may well be that these chemical stimuli act upon the same intracellular end apparatus as is affected by the nerve impulse.

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(47) LANGLEY: Journ. Physiol., 1898, xxiii, 240; 1904, xxx, 439.
(48) ERLANGER: Amer. Journ. Physiol., 1905, xiii, 372.
(49) SCHAFER: The endocrine organs. 1924.

THE FORMATION OF BILE PIGMENT

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THE SOURCE OF BILE PIGMENT.[1] The only known source of bile pigment is hemoglobin. Although no one has yet succeeded in preparing this pigment from hemoglobin *in vitro*,[2] there is convincing evidence that the living body is able to carry out the reaction. This evidence is in the form of very numerous clinical, morphological and experimental observations upon the association of bile pigment formation with red blood cell destruction, and of chemical studies which have demonstrated the intimate relationship between hematin and bilirubin.

The evidence that bile pigment can be formed from hemoglobin. In 1847, Virchow (105) published his celebrated discovery that a pigment having certain physical and chemical properties characteristic of bilirubin can frequently be found at the site of old blood extravasations. His studies led him to derive that pigment from the hemoglobin of the disintegrated red blood cells. This observation, which is today a commonplace of the autopsy room and has been repeatedly confirmed experimentally in studies to be described below, was the first important evidence in favor of the origin of bile pigment from hemoglobin. Virchow could not prove conclusively that the pigment formed under such

[1] The term "bile pigment" is used in this article to denote especially bilirubin, which is the chief pigment excreted normally by the human liver into the bile. In certain animals, biliverdin, an oxidation product of bilirubin, is the predominating pigment. Both bilirubin and biliverdin may occur together in the bile; and, more rarely, even other oxidation products, as well as hydrobilirubin, a reduction product of bilirubin, have been described as constituents of the bile of various animals under various conditions.

[2] While this paper was in press, there came to my attention a note by Parisot (Comptes Rendus de l'Académie des Sciences, 1911, cliii, 1518) in which he states that when laked blood is incubated *in vitro* with epinephrin, the hemoglobin becomes transformed into biliverdin. Parisot's experiment has been repeated, and it is quite true that, under such conditions, a green pigment resembling biliverdin does appear after some hours. We have not yet studied this pigment thoroughly enough to be certain of its actual nature.

circumstances is identical with bilirubin, and he therefore gave it the name of "hematoidin." Indeed, even at the present time there are writers who maintain that the "hematoidin" found in hemorrhages is quite different from true bile pigment (100) or at least that there is no proof that the two substances are identical (37). Of course, since we do not yet know the details of the chemical structure of bilirubin itself, we are unable to say with absolute certainty that bilirubin and "hematoidin" are identical; but they have, apparently, the same percentage composition (although, unfortunately, analyses have been made only upon material obtained from echinococcus cysts of the *liver* (22), (84)) and they are so much alike physically and chemically that most workers who have studied them have felt safe in the belief that bile pigment itself can be formed from hemoglobin locally in blood extravasations (Jaffe (39), Quincke (74), Stadelman (94), Hooper and Whipple (35), Van den Bergh and Snapper (103), Leschke (51), McNee (64)). The statements which are scattered throughout the literature concerning the failure of "hematoidin" to give a typical Gmelin test, or to form crystals typical of bilirubin, or to possess the solubility characteristics of bilirubin,—statements which have so often in the past disturbed the acceptance of the identity of these pigments (48), can very probably be referred either to the presence of loosely bound impurities in the "hematoidin" examined, or to some change of such a little-understood nature as that which is known to alter the properties of even gall-bladder bilirubin on standing (1). Rich and Bumstead (80) have subjected "hematoidin," obtained from old hemorrhages, to the long series of physical and chemical tests and reactions which are well established as characteristic of bilirubin, and in every instance the "hematoidin" behaved precisely as did a control of pure bilirubin. In this study it was found that "hematoidin" yields oxidation and reduction products (bilicyanin and urobilin (hydrobilirubin)) which have the same properties and are identical spectroscopically with the substances obtained by the same methods from pure bilirubin. It seems clear that in "hematoidin" we have to deal with a substance which is so much like bilirubin that it cannot be distinguished from the latter pigment by any of our present physical or chemical tests. The burden of proof must, therefore, rest upon those who may deny that true bile pigment can be formed at the site of blood extravasations.[3]

[3] In birds in which biliverdin is the predominant pigment of the gall-bladder bile, biliverdin (i.e., a bright green pigment which gives a positive Gmelin test) is formed in blood extravasations as well as "hematoidin." This is a further proof of the local formation of true bile pigment in hemorrhages.

Experimental evidence of the origin of bile pigment from hemoglobin began to appear about ten years after Virchow's discovery. Interest in the matter was precipitated by the experiments of Frerichs and Städeler (23) who found that a pigment resembling bilirubin could be produced *in vitro* by the action of sulphuric acid upon bile acids,[4] and, more important, that the injection of bile acids into the blood stream of an animal would be followed by the appearance of undoubted bilirubin in the urine. Their conclusion was that the body could transform bile acids into bile pigment. Kühne (44), shortly after, repeated and confirmed a forgotten or unnoticed observation of von Dusch (107) that bile acids are powerful hemolytic agents; and he insisted that the experiments of Frerichs and Städeler did not prove the origin of bilirubin from bile acids, for those investigators had not taken into account the fact that a large amount of hemoglobin is set free in the plasma by the injection of bile acids. Kühne was unable to satisfy himself that the injection of hemoglobin alone, in the absence of bile acids, would be followed by bilirubinuria, and he was forced to hold to the idea that the bile acids were necessary in some way for the formation of bile pigment. Herrmann (31), however, in 1859, was able to produce bilirubinuria at will by inducing intravascular hemolysis with injections of distilled water. This was the first clear demonstration that the simple liberation of hemoglobin into the blood stream may be followed by an increased output of bile pigment in the urine. Neither Naunyn (70) nor Steiner (96) could confirm Herrmann's results, and they opposed the conclusion that hemoglobin can be changed by the body into bile pigment. Their failure, as well as that of Kühne, is less difficult to understand now, for we have learned that the appearance of bilirubin in the urine after intravascular hemolysis depends upon a number of factors, and that the absence of bilirubinuria as determined by the Gmelin test, is by no means a proof that there has been no increased formation of the pigment. Tarchanoff (101), on the other hand, not only confirmed Herrmann's work but, with the use of bile-fistula animals, carried the proof of the relation of hemoglobin to bile pigment still further by demonstrating, for the first time, that the introduction of pure hemoglobin into the circulation is followed by a marked increase in the amount of bile pigment excreted by the liver. Stadelmann (95) confirmed this observation of Tarchanoff in a more care-

[4] Hoppe-Seyler (36) was able to show that this pigment did not really have the properties of bilirubin, and later Städeler (93) himself denied the identity of the two pigments.

fully controlled series of experiments, and it has since been established beyond question by numerous other investigators, using a variety of experimental animals and procedures, that the liberation of hemoglobin into the blood stream of an intact animal is regularly followed by an increased production of bile pigment which, according to conditions, may be eliminated by the liver or the kidneys, or partially retained in the plasma and tissues producing jaundice (Minowski and Naunyn (66), Gilbert, Chabrol and Bernard (25), Brugsch and Yoshimoto (14), McNee (63), Whipple and Hooper (115), van den Bergh and Snapper (103), and Rich (76)).

The *clinical* evidence of the relation of hemoglobin to bile pigment is to be found in the many different pathological states in which the condition of the liberation of an excessive amount of hemoglobin into the circulation is reproduced. In all of these maladies it is the rule that the formation of bile pigment is increased above the normal level and the pigment content of the feces, the urine and even of the plasma and tissues may be very high. These clinically familiar conditions include paroxysmal hemoglobinuria, malaria, blackwater fever, hemolytic septicemias, poisoning with any sort of hemolytic agent, intravascular hemolysis following transfusion with mismatched blood, hemolytic jaundice, and pernicious anemia (in which, regardless of any debate about the hemolytic nature of the anemia, free hematin has been found with regularity in the plasma (8)). Lepehne (50), Eppinger and Charnas (18), Jones and Jones (40), Wilbur and Addis (111), Andrewes (2), Sonnenfeld (92) and others have carried out systematic studies of the bile pigment variations in such clinical conditions.

Finally, it has been clearly shown that bilirubin is closely related *chemically* to hemoglobin. One may consult the reviews of this subject by Fischer (21), Thannhauser (99) and Küster (46) for details and for further references. Confidently expected for a long time, the demonstration of this relationship first appeared in 1897 when Küster succeeded in obtaining identical acid oxidation products (Hämatinsauren) from both hematin and bilirubin. Other oxidation and reduction split-products common to both hematin and bilirubin have since been discovered, and many other facts pertaining to the relationship of these two pigments have been amassed.

The derivation of bilirubin from hematin has been represented theoretically by the equation

$$\underset{\text{Hematin}}{C_{32}H_{32}N_4O_4Fe} + 2\,H_2O = \underset{\text{Bilirubin}}{C_{32}H_{36}N_4O_6} + Fe.$$

Although no such reaction has yet been carried out successfully *in vitro,* it is safe to prophesy that the preparation of bilirubin from hematin will eventually be accomplished by the chemist. As welcome and satisfying as this achievement will be, it is scarcely necessary to demand it as an essential part of the proof that bile pigment may be derived from hemoglobin. In the light of the established relationship between hematin and bilirubin, the observations which indicate that the living body is able to carry out the transformation are of much greater significance than any artificial preparation of the pigment *in vitro.* The preparation of bilirubin from hematin is now to be desired more for the light which it might throw upon the obscure reactions involved in the intermediary metabolism of hemoglobin.

Summarizing the evidence that hemoglobin furnishes a source of bile pigment, the facts are *1, that hematin and bilirubin are closely related chemically; 2, that hemoglobin spilled into the tissues almost anywhere in the body may be transformed, locally, into bile pigment ; and 3, that the presence of an excess of hemoglobin in the circulation whether introduced in pure form experimentally, or liberated during hemolysis in pathological conditions, is regularly followed by an increased formation of bile pigment.*

Are there sources of bile pigment other than hemoglobin? The above facts leave no doubt that hemoglobin may be regarded as one source of bile pigment, and at the present writing there is no proof that any other source exists. However, there has been a search for other sources. Whipple and Hooper (118), (119) have presented experiments and arguments which, they believe, are favorable to the view that hemoglobin is not the only source of bile pigment. Chief among their considerations are 1, experiments on dogs with bile fistulae in which the feeding of a diet rich in carbohydrates was followed by a great increase in the amount of bilirubin excreted by the liver; from this it was concluded that the liver cells must be able to make bile pigment from sources other than hemoglobin; and 2, the liver of a dog with an Eck-fistula does not excrete as much bilirubin as that of a normal dog; from this they argued that if hemoglobin were the only source of bile pigment, it would follow that there must be less daily blood destruction in Eck-fistula dogs than in normal dogs. This they do not think probable. They prefer to believe that the liver cells are able to make bilirubin from materials other than hemoglobin and that, because of the lowered hepatic function attendant upon the conditions of the Eck-fistula, they manufacture less bile pigment from these other substances than normally.

In regard to the first of these points, Rous, Broun and McMaster (89) have recently shown that if, in such experiments, the total bile be collected for study over periods of twenty-four hours, instead of during six- or eight-hour periods only, as Whipple and Hooper practised, a carbohydrate diet will be found to be without effect upon the production of bile pigment. The total amount of the pigment excreted during twenty-four hours is quite the same whether carbohydrate be administered or not. Inlow (38) has also reported that in his experiments with bile fistula dogs, a diet rich in carbohydrates had no effect upon the amount of bile pigment formed. The results of carbohydrate feeding, therefore, can not be regarded as proof of the existence of sources of bile pigment other than hemoglobin.

Regarding the Eck-fistula experiments of Whipple and Hooper, it may be said that although the liver of an Eck-fistula dog does apparently excrete less bile pigment than that of a normal dog, one must consider that by diverting away from the liver about three-fourths of its normal blood supply, profound alterations in pigment metabolism might be expected even on the view that hemoglobin is the sole source of bile pigment. Following the work of Brugsch and Yoshimoto (14) who reported that hematin injected intravenously could be recovered almost quantitatively as bilirubin in the bile, it became the fashion to compute the exact amount of daily red blood cell destruction under normal and pathological conditions from the quantity of bile pigment excreted in a given period of time. The more thorough investigations of Whipple and Hooper (115), and of Broun, McMaster and Rous (12), have shown clearly that no such refined and convenient calculations are justified, for hemoglobin is not promptly excreted quantitatively as bile pigment. The amount of bile pigment excreted usually falls below the quantity which a given amount of hemoglobin should yield theoretically. It is not certain whether the discrepancy is the result of a process of pigment conservation (although there is much in favor of this view), or whether some of the hemoglobin is broken down into substances other than bile pigment which are excreted without recognition. But whether excreted or conserved, it seems that the colorless leucoderivatives of hematin and bilirubin may play an invisible rôle in the katabolism of hemoglobin even under normal conditions, and it is clear that, aside from any question of variations in red blood cell destruction, one may have to reckon with variations in the relative amounts of bile pigment and of invisible leucoderivatives which will be formed from a given amount of hemoglobin in any series of experiments in which pigment metabolism

is disturbed, as it apparently is in Eck-fistula dogs. Whipple and Hooper themselves believe that exactly such a "deviation of pigment elements" can follow mere dietary changes (113). It would seem even more to be expected following such a metabolic insult as the combined Eck- and bile-fistula. Furthermore, when hemoglobin is injected into the portal vein it is said to be removed from the blood stream much more rapidly (85) (doubtless through the agency of the Kupffer cells), and a greater amount of bile pigment is said to be formed from it (6) than when the same amount is injected into a systemic vein. These points bear directly upon the conditions of the Eck-fistula. It seems, therefore, that hemoglobin may be normally converted by the body, at least in part, into substances which are at present unfamiliar but which are *not* bile pigment;[5] and, (although this point should be restudied with bile-fistula animals), that the longer the journey of a given amount of hemoglobin to the liver the more of such substances, and consequently the less of bile pigment, may be formed from it. Finally, although Whipple and Hooper are unwilling to believe that there may be less blood destruction in Eck-fistula animals than in normals, Pearce, Krumbhaar and Frazier (73) state that following the adminstration of hemolytic agents much less blood destruction occurs in Eck-fistula animals than in similarly treated normals. There are numerous little-understood factors involved in the Eck-fistula experiments of Whipple and Hooper which do not permit one to regard the conclusion drawn from them as the only possible one.

However, it must not be thought that we are certain that hemoglobin is the only source of bile pigment. One bears in mind in this connection the chemical similarity between chlorophyll and hematin (59) (Willstätter (112) has shown that ätioporphyrin may be obtained from either of these substances) which makes it conceivable that bile pigment could be synthesized from a portion of the chlorophyll molecule—or, indeed, for that matter, from any other pyrrol-containing substance ingested as food or liberated by body cells. And then there is the little-understood pigment of muscle which, conceivably, may be a source of bile pigment. The nature of this pigment is not yet clear. Some writers maintain that it is identical with hemoglobin and, indeed, it has many points in common with hemoglobin—such as its affinity for oxygen and the manner in which it combines with carbon monoxide.

[5] The writer has repeatedly observed that substances which are not bile pigment and yet do not give the Prussian blue reaction for iron, may be formed when hemoglobin is broken down by phagocytic cells.

Even the investigators who insist that myohematin is different from hemoglobin (notably, Günther (28)) admit that the difference between them may lie in the "globin" part of the molecule rather than in the pigment radicle (29); so that even though we possessed proof that myohematin could be a source of bile pigment, we would have to await proof that we are not after all dealing really with the hematin molecule itself. However, we have no evidence of the relation of muscle pigment to bile pigment. There are rare conditions in which the muscles are supposed to give up their coloring matter to the plasma in great quantity, becoming pale and often entirely colorless while a pigment similar to or identical with hemoglobin appears in the urine ("rheumatische kreuzlähme" of horses, and the so-called "myositis myoglobinurica" of man (29)). The occurrence of muscle pains and "hemoglobinuria" in soldiers after strenuous marches is also believed by some to represent a similar disturbance of muscle pigment metabolism. But these are conditions about which we know very little, and I have been unable to find any record of careful studies of bile pigment formation during such disturbances. McMaster, Broun and Rous (61) have shown that in dogs vigorous muscular activity may increase markedly the excretion of bile pigment, but they attribute this to the red cell destruction which Broun (11) found to be a result of muscular exercise. As for the transformation of ingested materials direct into bile pigment, Broun, McMaster and Rous (13) have shown that when cholohematin (a substance related to hematin, to chlorophyll, and to bilirubin) is ingested it is excreted, unchanged, into the bile; and Whipple and Hooper (120) have reported that the ingestion of hemoglobin (which we know can yield bile pigment) or, indeed, of bile pigment itself, does not bring about any increase in the output of bilirubin from a bile-fistula. It is, however, perfectly possible that pyrrol-containing substances derived from the food may be stored somewhere in the body and gradually utilized later in bile pigment formation. Whipple has constructed a schema to illustrate his ideas regarding this possibility (113); but it is fair to say that we have as yet no evidence of a compelling nature that food materials or any other substances can be used by the body in the formation of bile pigment unless they are first built up into hemoglobin.

Broun, McMaster and Rous (12) have recently restudied this question, using an ingenious technique which permits the aseptic drainage of the entire twenty-four hour output of bile, thus eliminating two important sources of error—infection of the biliary tract and short-period collections of the bile. They conclude that their data "support

the conception that bilirubin has no other sources besides the hemoglobin of destroyed blood."

THE LIVER AND BILE PIGMENT FORMATION. *Can the epithelial liver cells make bile pigment?* The fact that bile pigment is normally excreted by the liver has always been associated with the belief that the pigment is manufactured by this organ. It is true that Morgagni (68) and a few of the older writers taught that the liver merely excretes the bile brought to it, already formed, by the blood; but it is only in recent years that such a view has received serious examination and study. The result of this study has been that, from the vogue of the 1890's and early 1900's when scarcely anyone paused to doubt the status of the epithelial liver cell as the normal manufacturer of bile pigment, we have today swung to the opposite position of questioning whether the liver cells can actually make this pigment under any conditions. We may say at once that those who prefer an affirmative answer to this question have advanced only the most meagre and equivocal evidence in favor of it.

The question cannot be answered by morphological studies alone. It is obvious that the frequently discussed fact that bile pigment cannot be detected in the liver cells under normal conditions proves nothing beyond the inadequacy of our present histochemical methods; for, regardless of where it is made, since the pigment is excreted by these cells it must surely be present within them under physiological conditions. On the other hand, stress has often been laid upon the fact that bilirubin can be found within the tumor cells of primary liver-cell carcinoma, even in metastases outside the liver, and Lubarsch (55) goes so far as to say that this fact "establishes beyond all doubt the ability of the liver cell to produce bile pigment." However, as the writer has pointed out in another place (78), this phenomenon could certainly be explained equally well on the view that the liver cell has the power to remove bilirubin from the blood stream as a part of its excretory function, and it has been thoroughly well proven by Tarchanoff (101), Vossius (109), Wertheimer (110), Makino (56) and others that the hepatic cell does possess a remarkable power of taking up bile pigment from the blood stream.

The morphological study of the liver in acute yellow atrophy or following administration of such liver poisons as phosphorus, chloroform, carbon tetrachloride, etc., offers evidence against the epithelial liver cell as the site of origin of bile pigment, for in such conditions in which the liver cells may be destroyed in varying extent, it is well known

from clinical, pathological and experimental evidence that the more widespread the destruction of the liver cells becomes, the more intense is the jaundice which follows. This one would hardly expect were the bile pigment produced by the liver cells themselves. Indeed, in these same conditions the amount of plasma fibrinogen, a substance which is believed to be manufactured by the liver cells, is greatly diminished. Recently, Smyth and Whipple (91) have found that after such liver cell injury the formation of bile salts is greatly decreased, and they have used this as an argument in favor of the view that the liver cell produces the bile salts. It is obvious that the same reasoning applied to the continued formation of bilirubin in such conditions would lead to the conclusion that the liver cells do not produce bile pigment.

Previous to 1903, whenever it was suggested that perhaps the liver cells merely excrete the bile pigment which is made somewhere outside of them and carried to them by the circulation, it seemed rather easy to refute the suggestion by the statement that there is no bile pigment in the blood stream normally—not even in the portal vein. The very important demonstration by Gilbert, Herscher and Posternak (26) that normal human serum does contain bile pigment, removed this well-worn prop from the theory that the pigment is made within the liver cells. In 1913, van den Bergh and Snapper (104) applied Ehrlich's diazo-reaction to the quantitative study of bilirubin in the body fluids. This work really marked an epoch in the study of bile pigment under physiological and pathological conditions, for it placed in the hands of everyone interested in the matter a simple and extremely sensitive test for bilirubin in albuminous fluids.[6] With the van den Bergh test, the observation of Gilbert and his co-workers has been confirmed repeatedly, and it is now a perfectly familiar fact that bilirubin is normally present in human plasma in amounts which vary within fairly definite limits. If the quantity of plasma bilirubin be temporarily increased by injection of the pigment itself or by any other means which does not at the same time impair or overstrain the excretory function of the liver, the liver will promptly excrete the excess of pigment until the normal level of plasma bilirubin has been reached again. Bile pigment may, therefore, be regarded as a "threshold sub-

[6] Concerning the highly interesting and important problems associated with the nature of the "direct," "indirect" and "biphasic" forms of this reaction the reader is referred to papers by van den Bergh (102), Lepehne (50), Feigl and Querner (20), Makino (56), McNee (64) and Andrewes (1); and, also, in this connection, to the work of Blankenhorn (9).

stance" for the liver. The amount of bilirubin in the blood stream normally varies, within certain limits, in different individuals, and even in the same individual at different times; but the daily physiological fluctuations in any one individual remain within quite constant limits. In man the range of the normal "threshold value" has been established by many investigators (64). In certain animals such as the dog, the pigment has practically a zero threshold value, for not a trace can be detected normally in the plasma—not even by the van den Bergh test which is sensitive to one part of bilirubin in one and a half million. However, even in these animals we must believe that the pigment may be present physiologically in the plasma in amounts too small to detect, for it is not uncommon that bilirubin appears in the urine in experiments during which the pigment cannot be detected in the blood; and when its excretion by the liver is prevented, by the removal of that organ, the pigment may accumulate in the plasma until present in a detectable amount. The knowledge that bile pigment is present normally in human plasma and that it has the relation of a "threshold substance" to the liver is extremely important. It becomes very difficult to reconcile this fact with the theory that the epithelial liver cell makes the pigment; for if that were true we would have to imagine that the liver cell excretes a part of its product into the bile-canaliculi and also pours a constant amount of it in the opposite direction into the blood stream. Aside from any question of the meaning of such a whimsical procedure the matter would be very perplexing in the face of the well-established affinity of these cells for bile pigment injected into the circulation. On the other hand, the presence of bile pigment in the plasma, physiologically, offers very significant support for the view that the pigment is made outside the epithelial hepatic cells and is merely excreted by them in the manner of the excretion of any threshold substance by the kidney. Indeed, if one compares the retention of bile pigment (jaundice) in the conditions of liver cell necrosis which have been mentioned above, with the retention of, say, non-protein nitrogen in cases in which the renal epithelium is destroyed (e.g., corrosive sublimate poisoning) one is tempted straightway to the analogy that bile pigment is made somewhere outside the liver cells and that jaundice results because the damaged liver cells are no longer able to excrete it. When we come presently to the mention of the occurrence of jaundice in hepatectomized animals it will be seen that the analogy is entirely supported by facts.

The only experimental evidence of any suggestiveness concerning the ability of the epithelial hepatic cells to form bile pigment was the work of Whipple and Hooper (118), (119), which has been discussed above. These experiments were supposed to prove 1, that increased activity of the liver cells (produced by carbohydrate feeding) is accompanied by an increased production of bile pigment; and 2, that decreased activity of the liver cells (produced by the Eck-fistula) is accompanied by a decreased production of bile pigment. Objections to one conclusion drawn from these experiments by Whipple and Hooper have already been stated, and the same objections obviously apply to their other conclusion—that the liver cells can form bile pigment. The Eck-fistula experiments, however, invite close study in regard to the question of the relative importance of the passage of blood through the liver in normal bile pigment formation. In this section we have been considering only the ability of the *epithelial* liver cells to form bile pigment. We shall see presently that the *Kupffer* cells of the liver require perhaps even more careful attention in this regard, and it will become clear that any changes in the output of bile pigment which may follow such a decrease in the blood supply to the liver as the Eck-fistula brings about cannot be referred so casually to an effect upon the epithelial liver cells alone.

Can bile pigment be formed outside the epithelial liver cells? We have seen that there is at present no proof whatever that the epithelial liver cell is able to produce bile pigment, and we have encountered certain facts which would fit, perfectly, the view that these cells merely excrete the pigment brought to them by the blood stream. Let us now examine the evidence upon which is built our certainty that bile pigment can be formed outside of and independently of the liver cells.

In the first place, bile pigment can be formed from hemoglobin in blood extravasations almost anywhere in the body. We have already mentioned this phenomenon and we shall return presently to an examination of the mechanism by which it occurs. The fact itself is the important thing for the present consideration. Whether the liver cells are able to produce bile pigment or not, it is certain that it can be formed independently of those cells in hemorrhages. However, it is clear that even this knowledge sheds practically no light upon the *normal* site of formation of the pigment; nor does it answer the question whether hemoglobin liberated *into* the blood stream (as it is liberated, perhaps, in the process of physiological red cell destruction) can be

converted into bile pigment outside the liver cells. The obvious manner in which to learn the answer to this question is to study the formation of bile pigment in animals from which the liver has been removed.

Because of the difficulties encountered in the attempt to remove the liver from mammals, the early investigators made use of frogs and birds, in which the circulatory arrangement greatly simplifies the procedure. The first experiments of this nature were reported by Johannes Müller in 1844 (69). Müller removed the liver from frogs, and reported that at the end of four days after the operation the serum appeared normal and did not give a positive test for bile pigment. In 1850 Kunde (45) performed similar experiments. His frogs lived only four days and did not become jaundiced. He states that the serum at the end of this time had a greenish tinge (biliverdin?) but did not give a satisfactory test for bile pigment. Moleschott (67) in 1852 repeated the experiment, using over sixty frogs, some of which lived as long as three weeks after extirpation of the liver. In no case did a tissue jaundice occur. Although Moleschott's experiments are widely quoted in connection with the question of extrahepatic bile-pigment formation, his attention was really given to the bile acids rather than to the bile pigment. Alcoholic extracts of blood, muscle, stools, lymph and urine at the end of three weeks after hepatectomy did not give a positive test for bile acids. Regarding bile pigment he states merely that the alcoholic solutions did indeed have a greenish-yellow color (biliverdin?) but that this coloration was not strong enough to tempt him to carry out tests for bile pigment. Recently Makino (56) has carried out similar experiments and has observed no tissue jaundice in frogs living as long as five weeks after hepatectomy. He does not mention the color of the serum, and up to the present there are no experiments on record in which the serum of hepatectomized frogs has been tested for bile pigment after such a lapse of time.

Frogs are, however, peculiarly unsuitable subjects to use for the solution of the problem for, strangely enough, although there is an abundant formation of bile pigment normally in the frog, no tissue jaundice will appear in these animals even weeks after the ligation of the common bile duct (97), (53), (56). Stern (97), therefore, in 1885, made use of pigeons in similar experiments, for the circulatory advantages existing in amphibians are to be found in birds also. First Stern showed that bile pigment can be detected in the urine of pigeons within one and a half hours and in the plasma within five hours after tying the common bile duct. Then he found that if all blood vessels

going to the liver were ligated before the bile duct was tied, no bile pigment could be detected in either urine or plasma during experiments lasting even twenty-four hours. He concluded, therefore, as had the previous experimenters, that bile pigment is formed by the liver. A year later there appeared the renowned experiments of Minkowski and Naunyn (66) who found, briefly, that the intravascular hemolysis produced by arseniuretted hydrogen in normal ducks and geese is regularly followed by intense jaundice; in hepatectomized ducks and geese, however, jaundice did not appear after such hemolysis. Their conclusion was that the liver is the site of origin of bile pigment, and this conclusion was immediately adopted throughout the world without any reëxamination of the matter until nearly thirty years later when McNee (62) carefully repeated their experiments and confirmed their observation that no marked jaundice follows intravascular hemolysis in ducks and geese from which the liver has been removed. We shall return presently to a discussion of this work of McNee for it introduced a new conception into the problem of the site of origin of bile pigment.

The evidence acquired from all of the above experiments in which the liver was removed from amphibians or birds led, as we have said, to the conclusion that, in these animals, the liver is the normal site of origin of bile pigment. Whipple and Hooper (116) however, working with dogs, reported that after the exclusion of the liver from the circulation by certain ligatures, hemoglobin introduced into the blood stream would be rapidly changed into bile pigment—so rapidly, indeed, that an actual tissue jaundice might be visible within two hours. They concluded from this that the liver is not necessary for the conversion of circulating hemoglobin into bile pigment. Interestingly enough, this conclusion found as immediate and widespread an acceptance as had the opposite one of Minkowski and Naunyn, and the experiments were not repeated until ten years later when Rich (78) showed that the method of ligatures used by Whipple and Hooper to exclude the liver from the circulation does not really attain that end. India ink introduced into the circulation after tying all of the blood vessels ligatured by Whipple and Hooper, found its way into and out of the liver by vessels of which no account had been taken by the latter workers, and the ink particles so carried into the liver were readily phagocytized by the Kupffer cells of that organ. Makino (56) has since found that hemoglobin introduced into the circulation in experiments such as those of Whipple and Hooper finds its way into the liver and is phagocytized and broken down by the Kupffer cells. Although only a relatively

small fraction of the normal blood supply to the liver can circulate through it under these conditions, the experiments of Whipple and Hooper obviously could no longer be regarded as having proven the extrahepatic origin of the bile pigment appearing in their animals. In other experiments (78), Rich either completely extirpated the liver after establishing a head-thorax circulation, or else actually excluded it from the head-thorax circulation by appropriate ligatures, and in such animals hemoglobin introduced into the circulation was not transformed into bile pigment in experiments lasting from one to five and a half hours. The test used for the detection of bile pigment in the plasma was very much more sensitive than that employed by Whipple and Hooper, and yet, although six out of thirteen such animals lived longer than the time in which Whipple and Hooper had observed the appearance of a visible tissue jaundice, and although the operation for the exclusion of the liver by ligatures was much less severe than the intrathoracic operation used by Whipple and Hooper, not even a trace of bile pigment could ever be detected in the plasma of the dogs when blood was prevented from passing through the liver. This, together with the demonstration that the blood was not actually prevented from reaching the liver in Whipple and Hooper's experiments, seemed to justify the conclusion that the liver is necessary for the conversion of circulating hemoglobin into bile pigment, although it was felt that the Kupffer cells rather than the epithelial hepatic cells were probably the active part of the organ in this connection. McNee and Prusik (65), shortly after, published experiments in which the work of Whipple and Hooper had been repeated and the matter studied, besides, by means of the introduction of hemoglobin into a "heart-lung circulation." In the latter experiments in which no participation of the liver was possible, not a trace of bile pigment was formed from circulating hemoglobin in experiments lasting as long as six hours. Their experiments were offered in support of the results of Rich.

However, the matter was not yet settled. Mann (57) had devised a method for extirpating the liver from dogs without interfering with the circulation through the remainder of the body; and Mann, Bollman and Magath (58) reported that in such hepatectomized animals bile pigment appears in the plasma within three to six hours after removal of the liver, and increases in amount up to the death of the animal. The appearance of the pigment could be hastened by the intravenous injection of hemoglobin and would occur even in the absence of the abdominal viscera. The results of these experiments have since been

fully confirmed by Rich (79) and by Makino (56). Every reasonable source of error has been carefully controlled. The conclusion drawn by Rich (78) from experiments in which the circulation was greatly restricted was too sweeping. It is certain that if an active circulation be maintained through the entire musculo-skeletal system after complete extirpation of the liver and all abdominal viscera from a dog, the plasma, which immediately after the operation contains no demonstrable trace of bile pigment, may become faintly stained with this pigment several hours after hepatectomy; that an actual tissue jaundice may occur after the lapse of sufficient time; and that the intravenous injection of hemoglobin may increase, markedly, the amount of bile pigment formed.

Since the method of hepatectomy used by Mann, Bollman and Magath involves a great reduction of the blood supply to the liver (ligation of the portal vein) for a period of time before the organ is removed, there arose the possibility that this preliminary interference with hepatic function might have called into play a compensatory reaction on the part of extrahepatic tissues such as the spleen and bone-marrow, and that the bile pigment formed after the liver was removed might have been the result of such a pathological compensatory process. Such an objection has been raised by Rosenthal (85), and had to be considered in the light of what we know of the readiness with which the liver, spleen and bone-marrow can participate in compensatory reactions with regard to hemoglobin metabolism, and also in view of the disturbance of bile pigment formation which follows ligation of the portal vein in Eck-fistula animals. Rich (79) has controlled this matter by observing the formation of bile pigment in dogs after removal of the liver by a method which leaves the entire musculo-skeletal system within the circulation but involves no preliminary interference with the blood supply to the liver. It is clear, therefore, that there exists some mechanism outside the liver which, if it is not continually in operation normally, is certainly able to take over the function of bile pigment formation on immediate notice. The relative activity of this extrahepatic mechanism under normal conditions when the liver is in the circulation will be discussed below, as will, also, the negative results derived from the experiments on amphibians and birds and on dogs with a restricted circulation. The important thing for the present consideration is the fact that bile pigment can be formed and appear in the plasma entirely independently of any activity of the liver. The demonstration of this fact by Mann, Bollman

and Magath is, indeed, one of the most important contributions ever made to our knowledge of the formation of bile pigment. For many years the question of the possibility of such extrahepatic bile pigment formation, and of the occurrence of an "hematogenous jaundice," has been a physiological and pathological problem in the face of which one was always confronted with "ifs" and "buts," and left with a feeling of insecurity. For the first time it has been proven beyond a doubt and without a source of error that bile pigment may continue to be formed, even to the point of jaundice, in an animal from which the liver has been entirely removed. The matter is of the greatest import to the question of normal pigment metabolism, and of the pathogenesis of the non-obstructive jaundices.

THE SITE OF ORIGIN OF BILE PIGMENT. Since there is no shred of proof that the epithelial liver cells can make bile pigment, and, on the other hand, the certainty that the pigment can be formed independently of those cells, it becomes a matter of immediate interest and importance to inquire into the manner in which the bile pigment appearing in the plasma of an hepatectomized animal, or at the site of blood extravasations, has been formed. It is obvious that there are two possible mechanisms by means of which the formation of bile pigment could be effected: first, the pigment could be formed by the activity of certain cells; and second, there might be present in the plasma and tissue fluids something in the nature of an enzyme which could form bile pigment from hemoglobin extracellularly.

If the process is an intracellular one, what cells are concerned? Does there exist a specific kind of cell which alone has the function of forming bile pigment, or have many different kinds of cells this power? The fact that the pigment may be formed locally from hemoglobin spilled into almost any tissue of the body has not infrequently led to a rather vague notion that widely different types of fixed tissue cells in the immediate vicinity had effected the change. Thus, without the slightest histological evidence, it has been assumed that ordinary vascular endothelium (Jones and Jones (40)) and pleural mesothelium (35) possess this power; and it has even been suggested that it may be a property of "living protoplasm in general" to be able to form bile pigment from hemoglobin (Whipple and Hooper (35)). However, evidence that perhaps only one particular type of cell is concerned with bile pigment formation has been accumulating ever since the publication of a paper by McNee (62) in 1913, in which the status of the epithelial liver cell as the

site of origin of bile pigment was seriously questioned and a highly suggestive and interesting substitute was offered.

The relation of the "reticulo-endothelial system" to bile pigment formation. In 1889 Löwit (54) published a careful morphological study of the site of origin of bile pigment. His object was to examine the microscopical evidence for and against the then prevalent view that the pigment is formed by the epithelial liver cells. To this end he made minute studies of the liver, both of normal frogs and of frogs in which excessive intravascular hemolysis had been produced. In no case did he find anything requiring the interpretation that the epithelial liver cells could transform hemoglobin into bile pigment; but in the Kupffer cells of the liver sinusoids he often found, even in normal frogs, fragments of erythrocytes, hemoglobin, bile pigment and an iron-containing residue, sometimes all side by side in the same cell. In frogs in which intravascular hemolysis had been produced, there was a great increase in the number of Kupffer cells containing these substances. He found similar cells laden with the same materials in the spleen and bone-marrow and occasionally even in the heart's blood. Löwit's conclusion was that under normal and pathological conditions bile pigment can be formed from hemoglobin outside the epithelial hepatic cells within the phagocytic cells of the liver, spleen, bone-marrow and circulating blood. Minkowski and Naunyn (66) had also described such cells in the same situations and had stated, distinctly enough, their belief that the transformation of ingested hemoglobin into bile pigment could take place within them; but for no very good reason they laid much more stress upon the epithelial liver cells as the site of origin of bile pigment. While Löwit, on the other hand, stressed the importance of these phagocytic cells, he by no means denied that the epithelial hepatic cells could form the pigment.

Löwit's paper appeared very shortly after the famous publication of Minkowski and Naunyn. The conclusion of the latter writers that bile pigment is formed within the epithelial liver cells had become so universally and unquestioningly accepted that Löwit's work aroused scant notice. In the years which followed it was learned, especially through the investigations of Ribbert (75), Goldmann (27), Aschoff (3) and Kiyono (43), that the cells which Löwit and Minkowski and Naunyn had described possess certain morphological and functional properties in common which warrant their being considered somewhat as a distinct "tissue," widely distributed throughout the interstitial and

perivascular tissues of the body, but especially prominent in the liver, spleen, bone-marrow, lymph glands, adrenals, connective tissue and circulating blood. These specific phagocytic cells have been termed the "reticulo-endothelial system" by Aschoff, and apart from any question of the propriety of that term or of the actual origin and relationship of these cells, it is certain that the Kupffer cells of the liver, the clasmatocytes (or mononuclear wandering phagocytes) of the connective tissue, the monocytes (or "large mononuclear cells") of the blood, and the characteristic phagocytic cells of the spleen, bone-marrow and lymph glands have to be considered seriously as a system of cells of perhaps unsuspected importance in a wide variety of connections. It remained for McNee to break away entirely from the tradition established by Minkowski and Naunyn, and to ascribe the function of bile pigment formation to the reticulo-endothelial cells rather than to the epithelial hepatic cells.

It has been mentioned that McNee (63) repeated the experiments of Minkowski and Naunyn and confirmed their observation that a marked jaundice did not result from intravascular hemolysis in birds from which the liver had been removed. However, McNee found in birds phagocytic cells with contents similar to those in the cells which Löwit had described in frogs, and he stated his belief that jaundice failed to appear in hepatectomized birds not because of the loss of the epithelial liver cells, but because it is the reticulo-endothelial cells which are necessary for bile pigment formation, and when the liver is removed the Kupffer cells (which in birds constitute the bulk of the reticulo-endothelium) are removed with it.

Since the publication of McNee's work the question of the relation of the reticulo-endothelial system to bile pigment formation has been the subject of many experiments and of much discussion. Although it is by no means yet established as true, the idea is a very engaging one, for many things would be more easy to understand on such a view. In the first place, it would explain the fact that hepatectomized amphibians and birds do not develop jaundice after intravascular hemolysis, while hepatectomized dogs do; for most of the reticulo-endothelial system is known to be concentrated in the liver in the former animals, whereas the distribution of these cells in the spleen, bone-marrow and lymph glands of mammals is much more extensive. One would therefore expect the removal of the liver to affect bile pigment formation much more markedly in the one case than in the other. As a matter of fact, the removal of the liver from birds did not com-

pletely suppress bile pigment formation. In the experiments of Minkowski and Naunyn and of McNee small amounts of bile pigment appear, and one recalls also that the serum of the frogs in the experiments of Kunde (45) and of Moleschott (67) was said to have become greenish after hepatectomy. These traces of pigment have been explained away in various ways by various writers, but McNee suggested that the extrahepatic portion of the reticulo-endothelial system could produce small amounts of the pigment in birds and probably much more in mammals where there is present outside the liver many more of these cells than in birds. In this connection it may be said that in the dog and the human the spleen contains a relatively enormous mass of reticulo-endothelial cells and there is proof that bile pigment can be formed within this organ. In certain conditions the blood of the splenic vein contains much more bilirubin than that of the splenic artery or of other veins (van den Bergh and Snapper (103), Kaznelson (41), Rich and Rienhoff (83)); and further, perfusion of the spleen with laked blood is said to lead to the appearance of bilirubin in the perfusate within one hour (Ernst and Szappanyos (19)), whereas similar perfusion of a large capillary bed lined with ordinary endothelium (heart and lungs) is not followed by the transformation of the hemoglobin into bile pigment (McNee and Prusik (65)). McNee and Prusik (65) state that in an experiment in which the spleen was connected with a heart-lung circulation and perfused with hemoglobin, no bilirubin was formed. It would seem important that further experiments of this nature be carried out, and that perfusions of other tissues rich in reticulo-endothelial cells (bone-marrow) be compared further with perfusions of organs poor in such cells.

The fact that jaundice results from the widespread liver damage in acute yellow atrophy, chloroform poisoning, etc., a fact which, we have said, is difficult to reconcile with the view that the epithelial hepatic cell makes the pigment, would be entirely in accord with the view that bile pigment is formed by the reticulo-endothelial cells, for in such conditions it is the epithelial liver cell rather than the Kupffer cell which is primarily damaged. It is well known that the Kupffer cells are much more resistant to injuries in general than are the epithelial liver cells. One can find apparently normal Kupffer cells (often indeed laden with bile pigment) in the midst of necrotic liver cells in many conditions ranging from chronic passive congestion to acute yellow atrophy. Thus even if the bulk of the reticulo-endothelial cells were situated in the liver in man, the jaundice in such conditions would be

easy to understand on the view that these cells continue to form the pigment which the damaged epithelial liver cells are unable to excrete.

[redacted] the fact that hemoglobin can be converted into bile pigment in hemorrhages into the tissues or the serous cavities, would be easier to understand on the view that the reticulo-endothelial cells have the power to form bile pigment, for these cells, which are present in practically all of the tissues as well as in the blood stream, accumulate usually in great numbers about extravasated blood and greedily ingest the red blood cells. Virchow (105), who discovered the occurrence of bile pigment in hemorrhages, describes clearly the presence of these cells; Langhans (47) and others have found them in great numbers about experimental subcutaneous hemorrhages; Sabin, Cunningham and Doan (90) have followed their prompt migration into the serous cavities when blood is introduced and have described the avidity with which they ingest the red cells. Rich and Friedenwald have observed hordes of these cells wandering into the vitreous humor and engorging themselves with erythrocytes after the injection of blood into the posterior chamber of the eye; and the writer has found them in great numbers, filled with red cells, in the bloody fluid contained in a cyst of the omentum. This fluid had a bilirubin content many times greater than that of the blood. The importance of these cells in erythrophagocytosis under normal and pathological conditions will be referred to below.

Finally, the results of the hepatectomy experiments upon mammals, outlined above, would be easier to understand on the view that the reticulo-endothelial cells form bile pigment. For, whereas the transformation of hemoglobin into bilirubin did not occur in a strict head-thorax circulation, if the remainder of the musculo-skeletal system alone were added to the head-thorax circulation bile pigment was formed and appeared in the plasma (79). That means that when hemoglobin was allowed to circulate through a larger mass of muscles and bone-marrow, it was transformed much more readily into bile pigment. It is, of course, possible that the muscle, or the vascular endothelium, is in some way the active agent, but the adherents of the reticulo-endothelial theory prefer to think of the bone-marrow in this connection (5).

If the reticulo-endothelial cells are the makers of bile pigment it should be possible, theoretically, to stop the formation of bile pigment by paralyzing these cells in some way. This was attempted in 1918 by Lepehne (49), who injected collargol intravenously in the effort so to overburden the reticulo-endothelial cells with that substance, which

they phagocytize voraciously, that they would be unable to carry out their supposed bile-pigment producing function; and, indeed, the animals (pigeons) so treated developed no jaundice at all or only slight and delayed jaundice when they were afterward subjected to poisoning with arseniuretted hydrogen—a drug which regularly produces hemolysis and jaundice in normal pigeons. Rosenthal and Melchior (87), Rosenthal and Fischer (86) and Bieling and Isaac (7) were unable to confirm this result. In their experiments jaundice followed either hemolysis (produced by toluylenediamine) or obstruction of the common bile duct in collargol treated animals (pigeons and dogs), and they conclude, therefore, that there is no proof that the reticulo-endothelial cells can form bile pigment. More recently Elek (16) has reported that, in dogs, the saturation of the reticulo-endothelial cells with intravenously administered colloidal iron decreased the bile pigment output from a biliary fistula to zero, while the total fluid output was not diminished. He believes that his experiments support Lepehne's conclusion that these cells can be paralyzed and made unable to form bile pigment. However, if one examines Elek's protocols it will be seen that during his experiments bilirubin was present in the urine and even in the plasma of his animals. Since bile pigment can never be detected in these fluids in the dog under normal conditions, one finds it difficult to correlate Elek's conclusions with the presence of a plasma jaundice. The writer carried out experiments exactly similar to Elek's several years ago. In the first dog the administration of colloidal iron was promptly followed by a most remarkable decrease in the day to day pigment content of the fistula bile; but in a second and a third dog, even after greatly increased doses of the colloidal solution over a longer period of time, the pigment output was not appreciably altered. (It may be stated in this connection that infection of the biliary tract in bile fistula animals may lead to a disappearance of pigment from the fistula bile (61).) It remains doubtful whether all of these cells can be so completely stuffed with colloidal materials that they will become quite unable to function. If this were possible one might well expect the cells to be unable to live under such conditions; and yet microscopically they appear normal except that their nuclei may undergo division, and that their cytoplasm usually increases in proportion to the amount of matter which they are forced to ingest—a fact which in itself must interfere somewhat with the attempt to fill them completely. The results of different investigators are at variance in regard to the question whether it is possible to fill these cells so completely with one

colloid that they will be unable to take in any other. It is true that Bieling and Isaac (7) and Gay and Clark (24) have recently reported that they could almost entirely prevent antibody formation by filling the reticulo-endothelial cells (which are receiving considerable attention in connection with immunity reactions also) with such colloids; but from the data available at present it can be said only that the attempts to paralyze these cells have proven nothing whatever about their bile pigment forming potentialities.

The view that the reticulo-endothelial cells are the site of origin of bile pigment is at present represented especially by Aschoff (4) and Lepehne (49) in Germany, by Eppinger (17) in Austria, and by McNee (64) in England. Certainly these cells play a visible rôle in the katabolism of hemoglobin in the lower animals and in man. It is well known that erythrophagocytosis is carried out practically exclusively by cells of the reticulo-endothelial variety. In lower animals, in which whole red blood cells are phagocytized normally in great numbers, it is these cells alone which are active in this work (42), (88). In higher animals and in man it is again the reticulo-endothelial cells which can be found performing the function of phagocytizing red cells and their fragments under normal and pathological conditions. Direct observations as well as microchemical stains for iron leave no doubt that the hemoglobin of the engulfed erythrocytes is broken down within these cells into simpler substances. It is not difficult to believe that cells which play such a rôle in the katabolism of hemoglobin throughout the animal kingdom may be the cells concerned in the formation of this katabolic product of hemoglobin—bilirubin. However, the reticulo-endothelial system is being ridden a bit hard just now. One authority (5) has recently written of these cells as important in the formation of bacterial immune bodies and other antibodies; in the metabolism of iron, of hemoglobin, of lipoids and of proteins; and in various proliferative defense reactions. Although there is proof of some of this and more or less evidence for the rest of it, one is perhaps justified in regarding this system of cells with the suspicion which any panacea provokes; but we can say, as far as their relation to bile pigment formation is concerned, that there is no observation on record in which bile pigment has been formed in the absence of cells of this type; that morphological and histochemical studies are strongly suggestive of the ability of these cells to make bile pigment from hemoglobin; that there is no such evidence pointing to the activity of any other type of cell in this connection; and, finally, that we have proof that the pigment

can be formed within one of the organs richest in reticulo-endothelial cells (spleen).

Against the reticulo-endothelial cells as the site of origin of bile pigment it has been urged (87) that, since these cells are very active phagocytes, the finding of bile pigment and iron-containing pigment by the side of hemoglobin within them in the experiments of Minkowski and Naunyn, Löwit and McNee, does not prove that the former pigments were made from hemoglobin inside the cells, for all three substances could have been phagocytized from the blood-stream. Similarly, most investigators who have studied the origin of bile pigment in hemorrhages have concluded that these cells, although present in large numbers at the site, have not the power to form bile pigment, and they have explained the occurrence of bile pigment within them by assuming that it had been phagocytized. This has been used as a rather powerful argument against the theory (55). It is obvious that the formation of bile pigment from hemoglobin in hemorrhages should offer an excellent opportunity to study not only the activity of this particular type of cell in this connection, but also to investigate the validity of the widely accepted assumption that bile pigment may be formed extracellularly by enzyme-like action. Numerous investigators have accordingly attempted to follow microscopically the fate of hemoglobin under such circumstances.

The mechanism of the formation of bile pigment in blood extravasations. Virchow (105) in 1847, first described the presence of "hematoidin" in old blood extravasations. It occurred in the form of characteristic crystals and in amorphous masses both within cells and lying free outside of cells. There was present also, within and outside of phagocytic cells, an iron-containing pigment (hemosiderin), and Virchow concluded that the hemoglobin of the extravasated red cells had been broken down into these two pigments. We have already considered the question of the identity of "hematoidin" with bilirubin, and the term "bile pigment," or "bilirubin," will be used freely in this connection throughout the following discussion. Virchow, studying the end result of the process in old hemorrhages found at autopsy, concluded from the distribution of the pigments that both hemosiderin and "hematoidin" could be formed both intra- and extracellularly.

More than twenty years elapsed before any further study of importance was applied to the matter. In 1870, Langhans (47) turned from the examination of old blood extravasations to the experimental production of hemorrhages in animals in the effort to study the early

stages of the process. He made the very important observation that wandering phagocytic cells (of the type now regarded as part of the reticulo-endothelial system) soon surrounded a mass of blood injected subcutaneously, and began to ingest the red cells. Later these phagocytes were found to contain clumps of the iron-containing pigment, hemosiderin. It was only rarely that they contained rhomboid crystals of bilirubin, which were to be found, however, in large numbers lying free outside of cells. He believed that the rhomboid crystals of bilirubin occasionally found within the phagocytic cells must have been phagocytized pre-formed. He was, however, less certain about the needle-shaped crystals of bilirubin, for he did find those within phagocytic cells even as early as the second day after injecting the blood. Langhans also noted the appearance of a diffuse, non-granular, green pigment which, he suggested, might be biliverdin and which he believed to be formed from hemoglobin outside of cells, although he regularly found it also within the wandering cells. He drew attention, further, to an important fact which has been repeatedly confirmed since—that bile pigment is to be found especially in the edge of the blood clot adjacent to the living cells of the tissues and never in the very center of the clot. Langhans' conclusion from his studies was that the ingestion of red cells by the phagocytic cells is followed by the intracellular transformation of the hemoglobin into hemosiderin, and that bilirubin is formed only when hemoglobin is broken down outside of cells in the tissue fluids.

In 1884, Quincke (74) carried out similar experiments and arrived at similar conclusions. He was, however, very much impressed by the fact that crystals of bile pigment were to be found only near living tissue and he felt that the change from hemoglobin to bile pigment required, in some way, the activity of living cells of the connective tissue. However, since he, too, found many of the crystals outside of cells, he was doubtful about the intracellular origin of all of them.

Four years later, Neumann (71) reported experiments on this question which were carried out so thoroughly and led to such dogmatically formulated conclusions that they have been regarded as final ever since. He, too, studied the fate of hemoglobin in blood injected subcutaneously into animals; and he drew a sharp line between the sites at which bile pigment and hemosiderin are formed. As Langhans and Quincke had found, he, too, found wandering phagocytic cells laden with hemosiderin about the margin of the injected blood mass; but crystals of bile pigment, although found occasionally within these cells, were present

in greatest abundance lying free within the clot in a zone just inside the zone of living hemosiderin-containing cells. Neumann concluded that whereas hemosiderin is formed only in regions where living cells are active in breaking down the hemoglobin, bile pigment will be formed only from hemoglobin which disintegrates entirely out of contact with living cells. "Hematoidin formation," he writes, "is a chemical metamorphosis independent of vital tissue activity." (It should be mentioned, however, that he, too, noted that in the center of the blood mass—the part furtherest removed from vital tissue activity—crystals of bile pigment were never to be found.) As an argument in favor of his conclusion Neumann writes that "in cases in which the extravasated blood is distributed diffusely in the tissues and a mass of blood clot does not exist, as for instance in small blood extravasates (capillary hemorrhages and diapedesis hemorrhages) hematoidin formation never does occur." We shall have occasion to refer again to this widely accepted dictum.

In the same year in which Neumann's publication appeared, his pupil Skrzeczka (98) reached the same conclusions from similar experiments; and ever since, in practically all monographs and text books, the matter is represented according to Neumann's view, i.e., when hemoglobin is phagocytized by clasmatocytes bile pigment is never formed—only hemosiderin; when hemoglobin disintegrates in the tissues out of the reach of living cells, then only will bile pigment be formed. We have mentioned that this conclusion has been widely used as an argument against the ability of the reticulo-endothelial cells (here represented by the clasmatocytes and blood monocytes) to form bile pigment.

There are a number of very good reasons for examining thoroughly the statement that hemosiderin is formed only by living cells in one zone and bile pigment in a different region uninfluenced by the activity of living cells. It will suffice here to point out in the first place that whenever iron-free bilirubin is split off from hemoglobin, an iron-containing residue must be present at the same site. Furthermore, most of the above investigators have considered only *crystalline* bile pigment and have entirely neglected the fact that bilirubin will be overlooked when present in cells in *soluble* form in amounts too small to be detected microscopically by the Gmelin test. In mammals, bilirubin does not so readily crystallize out in living cells or in the alkaline body fluids. In birds, on the other hand, crystals of bile pigment form much more readily in the cells and fluids, and the only investigator who has thor-

oughly studied the matter in birds (Langhans) was impressed by the frequency with which needle-shaped crystals of bilirubin could be found within clasmatocytes very shortly after the phagocytosis of red cells had been begun. Finally, when one injects blood subcutaneously, and then, removing it for study after a lapse of time, finds crystals both within and outside of cells, it becomes largely a matter of individual interpretation whether one assumes that the crystals which are found were all formed intracellularly and some subsequently liberated after disintegration of the cells, or that all crystals were formed outside of cells and when found intracellularly merely represent phagocytosis. It will be recalled that, in the face of this dilemma, Virchow chose to believe that both interpretations were admissible.

It is obvious that if red blood corpuscles could be kept under continuous observation after being ingested by clasmatocytes, or while disintegrating in tissue fluids outside of living cells, a much clearer idea of the process might be obtained. This Rich (77) has tried to do by the use of the Lewis method of tissue culture. Fresh red blood cells were added to cover glass cultures containing wandering phagocytic cells of mesodermal origin, and the fate of the red cells was followed under the microscope. It was found that the phagocytes wandered among and ingested the red cells in large numbers, and that, as the hemoglobin was broken down within the phagocytic cells, beautiful rhomboid or needle-shaped crystals typical of bilirubin, and often diffuse, bright green biliverdin appeared within the cytoplasm of the living phagocytes. These pigments gave a perfect Gmelin test under the microscope and possessed every other chemical and physical property of bile pigment for which they were tested. The Prussian blue reaction often showed the presence of an iron-containing residue within the phagocytic cell by the side of the bile pigment. Up to the stage in which crystals of bile pigment first appeared within the phagocytes, no crystals were ever found outside of these cells, although there were often great numbers of red cells disintegrating extracellularly. Later, as the clasmatocytes themselves began to die and to disintegrate, the intracellular crystals and soluble bile pigment were, of course, set free; so that in older cultures there might be numerous crystals lying outside of cells some of which apparently formed extracellularly from soluble bile pigment. It was certain, however, from the study of hundreds of such cultures that the hematoidin crystals can make their first appearance *within* phagocytic cells, and that they were not phagocytized

preformed. Cultures of cells of ectodermal or endodermal origin remained entirely indifferent to red blood cells, and no bile pigment was ever seen when red cells were added to dead cultures of even phagocytic cells, but the intracellular crystallization of bile pigment occurred in practically every culture in which red blood cells were phagocytized by clasmatocytes from any part of the body. Indeed, even in a drop of bird's blood incubated alone, the monocytes (which are also a part of the reticulo-endothelial system, it will be remembered) would frequently ingest the surrounding red cells, and this might be followed by the appearance of bile pigment and an iron-containing residue within the body of the monocyte.

No prettier pictures of the process as observed in tissue cultures can be desired than those of the Kupffer cells in the older papers of Löwit (54), of Minkowski and Naunyn (66), and of McNee (63). The experiments are, however, not yet entirely free from the possibility that bile pigment may have been present invisibly in soluble form in the tissue fluids, and until this possibility of phagocytosis of the pigment in soluble form from the surrounding medium can be controlled, it cannot be stated with any certainty that hemoglobin can be converted into bilirubin within phagocytic cells of the reticulo-endothelial variety. However, the experiments do show clearly enough that the statement (71), (55), that crystalline bile pigment never occurs in small diffuse extravasations in which the red cells are engulfed by phagocytes (i.e., in hemorrhages where there is no circumscribed blood mass in which hemoglobin may disintegrate out of the reach of living tissue) is not really true; for the appearance of crystalline bilirubin has been observed repeatedly in cultures containing only a very few red cells, if those cells were ingested by clasmatocytes. In this connection, the writer has recently had occasion to study a series of human cases at autopsy, in which minute capillary extravasations of blood were found in the meninges—small, diffuse scatterings of red cells in which no dense blood mass formed. The cases ranged from very fresh hemorrhages in which the red cells were still intact, to later stages in which the filmy vascular membrane familiar in pachymeningitis hemorrhagica was present. A study of these little extravasations shows clearly that clasmatocytes quickly accumulate in great numbers, and wander among and ingest the red cells; and one can hardly escape the impression (although here again, we have merely an individual interpretation) that the plentiful intracellular rhomboid and needle-shaped crystals of bilirubin were

formed *within* these phagocytic cells by the side of hemosiderin, just as in tissue cultures. The meninges appear to be an exceptionally favorable site for the crystallization of bile pigment within living cells.

Certainly, as one follows the process in tissue cultures, there does not appear to be the slightest foundation for the assumption that bile pigment and the iron-containing substance always appear at different sites, respectively. In the early stages both are found within cells. Later, when cellular disintegration is in process, both may be found outside of cells. What then is the explanation of the opposite conclusion of the earlier investigators, and of the fact that in old hemorrhages discovered at autopsy with the mentioned exception of small meningeal extravasations, bile pigment crystals are found most frequently outside of cells? The answer to this question is linked with the problem of the conditions which determine where and when bilirubin will *crystallize* out of solution within the living body; and this problem is a very perplexing one. The presence of crystals of bile pigment within living cells is a phenomenon which, as far as I have been able to find, does not occur under normal conditions in any animal higher than Amphibia. In both birds and mammals it occurs only under pathological conditions, but much more readily in birds than in mammals. Although it is certain, from the tissue culture experiments, that the crystals can be formed within living cells, it is also entirely probable that in hemorrhages some of the bile pigment formed may crystallize out extracellularly, for it is clear from the hepatectomy experiments and from the analyses of the splenic vein blood that if bile pigment be manufactured intracellularly, whether within the reticulo-endothelial or any other type of cell, it is promptly discharged in soluble form into the blood stream to be excreted by the liver; in hemorrhages also, then, if hemoglobin is converted into bile pigment intracellularly, some of the latter pigment would be expected to be discharged from the cells, and such liberated soluble bile pigment would tend to diffuse into and to crystallize out in that part of the hemorrhage which offered the most favorable conditions. The fact that necrotic tissue tends to imbibe bile pigments (37) might serve to explain the concentration of the pigment at the margin of a hemorrhage where autolysis is most prominent; and since bilirubin is quite soluble in slightly alkaline solutions and insoluble in acid ones, a factor favorable to crystallization of the pigment may be the reaction of the medium. Bilirubin has also a curious tendency to crystallize out in fat droplets. However, these are merely isolated observations and can by no means

entirely explain the matter of the crystallization bile pigment in living cells and tissue fluids.

There is a possibility in connection with the formation of bile pigment in hemorrhages which cannot be passed over without serious consideration; it is entirely possible that bile pigment is formed from hemoglobin outside of and independently of the activity of cells, and that it appears at first in the fluids in soluble, and therefore invisible form, becoming visible only when it has been concentrated within the phagocytic cells which may ingest it as they ingest other colloids. One may point out that the same possibility applies to hemosiderin as well. This brings us to the consideration of the second, or extracellular possibility mentioned some pages back, i.e., that there may exist extracellular enzymes which have the power to convert hemoglobin into bile pigment.

The question of the formation of bile pigment by extracellular enzymes. This question, although one of immediate importance, has received very scant attention in spite of (or perhaps because of) the confident assurances in practically every textbook that when bile pigment is formed in hemorrhages it is always formed extracellularly. These assurances are based entirely upon the morphological studies of hemorrhages which have been outlined above.

Years ago von Recklinghausen (108) reported that when whole frog's blood was kept under aseptic conditions the clear serum became green (biliverdin?) after several days, and that he then found hematoidin crystals within the white blood cells. This experiment has been regarded by some as a demonstration that hemoglobin undergoing sterile autolysis may be converted (by a plasma or cellular enzyme?) into bile pigment independently of the activity of living cells. By others it has been regarded with skepticism, and the crystals seen by von Recklinghausen have been supposed to have been hemoglobin crystals (37). Neither Leupold (52) nor Leschke (51), nor Hueck (37) could confirm the observation, using human blood. I have found no recorded attempt to repeat the experiment with the blood of amphibians or birds, in which bile pigment crystallizes out much more readily. Since we have seen that, in the tissue culture experiments (77), bile pigment may make its appearance in the form of crystals within the living large mononuclear cells in sterile cultures of pure bird's blood, von Recklinghausen's observation is confirmed; but his experiment cannot be considered as one from which the activity of living cells had been eliminated. Indeed, von Recklinghausen himself believed that the crystals of bile

pigment had been formed from ingested hemoglobin by the white blood cells. In Rich's experiments the pigment was never seen to appear in cultures of blood in which there were no living white cells, not even in sterile cultures kept for weeks.

Crofton (15) has reported that if the enzyme trypsin be added to a solution of hemoglobin, bile pigment will be formed when glucose is also added to the mixture. Hollis (34) reports confirmation of this observation but says that glucose is not necessary for the transformation of hemoglobin into bile pigment by the action of trypsin. He states further that the more air bacteria there were in the experimental fluids, the more bile pigment was formed; and, indeed, that the hemoglobin of laked blood left standing alone exposed to the air for some days was converted into bilirubin. Herzfeld and Steiger (32) have reported that the Fränkel pneumococcus has the power to convert hemoglobin into bile pigment within three days. Leupold (52), on the other hand, was not able to observe the formation of bile pigment from hemoglobin exposed to bacterial action.

Rich and Bumstead (81), (82) have repeated the experiments of Crofton, Hollis, and Herzfeld and Steiger, using the van den Bergh and Huppert-Salkowski tests for bile pigment, both of which are much more sensitive than the Gmelin test. In repeated experiments, laked blood as well as crystalline hemoglobin was exposed to the action of 1, trypsin, 2, trypsin and glucose, 3, air bacteria, 4, the Fränkel pneumococcus, 5, different strains of streptococcus viridans, and 6, staphylococcus aureus. In no instance was a trace of bilirubin detected in tests made frequently during the period of a week. It is very interesting and pertinent that with the Gmelin test, which was the one used by Crofton, Hollis, and Herzfeld and Steiger, a bright green ring often appeared at the junction of the nitrous acid and the fluids tested and, after the action of trypsin, above this a reddish zone might appear; but in view of the entirely negative van den Bergh and Huppert tests, it was clear that these colors were not due to the presence of bile pigment. The full play of colors of the Gmelin test was never seen. Fresh hemoglobin does not give this reaction but when hemoglobin is allowed to stand for a number of hours under widely different conditions this atypical Gmelin reaction may occur, and unless the appearance of the colors be carefully watched for it might easily be mistaken for a positive test for bilirubin. The sediments of the above solutions were searched for bilirubin by extraction with chloroform and by microscopic examination for crystals, with equally negative result.

One is, however, not so much interested in the action of bacteria and trypsin upon hemoglobin as in the question of the existence in the body fluids of an extra-cellular enzyme which has the power to form bile pigment. The only description (and that a very meagre and incomplete one) of a search for such an enzyme which I have been able to find has been a single experiment by Leschke (51), which, he believed, demonstrated the presence of a bile pigment forming enzyme in the spinal fluid after the injection of blood. Aside from numerous objections to Leschke's experimental method, the result of his experiment has not been confirmed (81). Rich (77) attempted to examine the matter in tissue cultures by adding red blood cells and laked blood to freshly dead, sterile cultures of various tissues and also to extracts of tissues. The formation of bile pigment was never seen under these conditions. However, since the pigment might have been present invisibly in solution, Rich and Bumstead (81) have studied the question in a manner which permitted chemical tests for bile pigment to be applied to the solution. As we have pointed out, the spleen has the power to form bile pigment under certain conditions. Fresh spleens, therefore, were ground up under aseptic precautions and extracted with sterile saline or distilled water. After centrifugalization, portions of the supernatant fluid, mixed with laked blood, were buffered through a wide pH range and allowed to stand at body temperature. Frequent tests (van den Bergh, Huppert, Gmelin, extraction with chloroform) were made during the period of a week, but in no case could a trace of bile pigment be detected in either the supernatant fluid or the sediment. In other experiments the spleen was cut into small pieces, mixed with laked blood under aseptic precautions and allowed to autolyze at body temperature, with a similar negative result. Aside from the question of bile pigment forming enzymes, such experiments indicate that the mere presence of autolyzing body cells is not sufficient to cause the formation of bile pigment from hemoglobin, as has been stated by Hauser (30). Hauser's experiments are often referred to in this regard, but they were really carried out for an entirely different purpose and no attempt whatever was made to ascertain whether the "needle-shaped crystals" which he saw in blood exposed to autolysing tissues were actually bile pigment.

If the contention of those who plead for a "humoral" or enzyme formation of bile pigment in the circulating blood or tissue fluids were true, one would certainly expect the enzyme to be present in the plasma. However, the hemoglobin of sterile whole blood adjusted to different

degrees of acidity and alkalinity and kept in a thermostat, was not converted into bilirubin nor could an enzyme be found in the plasma after injected hemoglobin had been allowed to circulate in the blood stream for an hour (81). In this connection, Makino (56) tied off from the circulation one leg of a dog from which he had removed the liver, and although bile pigment appeared in the general circulation after some hours, the plasma in the tied-off limb remained free from the pigment. He concluded from this that the formation of bile pigment is not dependent upon the presence of an enzyme in the plasma. Oppenheimer (72), in this laboratory, had previously carried out the same type of experiment in animals in which intravascular hemolysis had been produced before the limb was isolated from the circulation, and had found that no bile pigment was formed from the hemoglobin in the vessels of the tied-off member. Hooper and Whipple (35), also, have stated, without describing their method of search, that they could not demonstrate a bile pigment forming enzyme in tissue fluids or extracts. Thus, up to the present, the few efforts which have been made to demonstrate the existence of an extracellular enzyme capable of forming bile pigment have been without success. The demonstration of such an enzyme would be a matter of fundamental importance to the subject of hemoglobin metabolism, and there are enough facts suggesting the possibility of an extracellular mechanism of this nature to warrant the most careful and persevering attempts on the part of different investigators to determine whether or not one exists. This is, perhaps, the most urgent problem connected with the question of the site of origin of bile pigment today—a problem about which much has been said and very little done. Since Virchow (105) first drew attention to the possibility, writers have not ceased to discuss the "humoral" transformation of hemoglobin into bile pigment within the circulating blood. Indeed, we have even been given hypotheses concerning the origin of the hypothetical enzyme (5). But systematic attempts to determine whether there really is such an enzyme have been scarcely begun. In the light of the proof of the formation of bile pigment in hemorrhages, in the spleen and in animals from which the liver and all abdominal viscera have been removed, it now appears most probable that the answer to the problem of the site of origin of bile pigment lies either in the reticulo-endothelial cells or in this, as yet hypothetical, enzyme.

The normal site of origin of bile pigment. Can any conclusions be reached as to the normal site of origin of bile pigment from a considera-

tion of the various studies outlined above? Since the pigment continues to be formed after removal of the liver and all of the other abdominal organs, one is forced to consider besides the reticulo-endothelial system and extracellular enzymes, the possibility of its formation by *ordinary vascular endothelium*. Whipple (114), Jones and Jones (40) and others have ascribed the power of bilirubin formation to ordinary vascular endothelium and on such a view hemoglobin circulating through the blood vessels anywhere in the body could be changed into bile pigment. The latter investigators produced local intravascular hemolysis in one arm of a paroxysmal hemoglobinuric by immersing it in cold water after placing a tourniquet about the upper arm. They reported that twenty minutes after hemolysis had been induced the plasma from the vessels of the isolated member had a "strong reddish tinge" because of the presence of free hemoglobin, and gave a negative Gmelin test; then, within the amazingly short time of thirteen minutes, the next specimen of plasma withdrawn from the arm was found to be "dark golden yellow" and it gave a positive Gmelin test (i.e., hemoglobin had become changed into bilirubin). Neither hemoglobin nor bilirubin (Gmelin test) could be detected in the blood of the systemic circulation during this time. This experiment has been reproduced in dogs in all important details by Oppenheimer (72), but hemolyzed blood remaining for over six hours in the vessels of a limb excluded from the general circulation was not transformed into bilirubin (van den Bergh test). A similar experiment by Makino (56), also with negative result, has already been mentioned. Jones and Jones reported only a single experiment of this nature. It seems clear, as Oppenheimer has pointed out, that if their observation is confirmed in other cases (the van den Bergh quantitative method should be used) there must be either a marked difference between the bilirubin forming potentialities of the vascular endothelium of the paroxysmal hemoglobinuric and the normal human, or else a difference between the endothelium of dog and man in this regard. In any event the experiment of Jones and Jones offers no proof that the formation of bilirubin was effected by the activity of the vascular endothelium. The experiments of Oppenheimer and of Makino, as well as those of McNee and Prusik (65), speak against the bile pigment forming power of ordinary vascular endothelium. The latter workers perfused laked blood and hemoglobin through isolated "heart-lung circulation" preparations, and not a trace of bile pigment was formed during six hours (van den Bergh test). The evidence seems to be against the view

that ordinary vascular endothelium takes a part in the formation of bile pigment.

The *spleen* had often been referred to as playing an important rôle in the normal formation of bile pigment—sometimes as a site of origin of the pigment, sometimes as an organ which "prepares" the hemoglobin of disintegrated corpuscles for its conversion into bilirubin by the liver or elsewhere. It has already been mentioned that in certain conditions such as pernicious anemia and hemolytic jaundice, the splenic vein may contain much more bilirubin than the splenic artery or peripheral veins (103), (41), (83), and this must be interpreted to mean that bilirubin is being formed within the spleen in these conditions. However, although it seems clear from these observations that bile pigment can be formed within the spleen under certain pathological conditions, Rich and Rienhoff (83) found no difference between the bilirubin content of the splenic vein and peripheral veins in conditions in which hemoglobin metabolism was normal, nor in acute hepatitis with jaundice, or cirrhosis of the liver. Further, whereas van den Bergh and Snapper (103) found an elevated bilirubin content of the splenic vein in the experimental hemolytic jaundice produced by phenylhydrazine, they did not find this to be true in the hemolytic jaundice produced by toluylenediamin; nor did Rich (76) find any appreciable difference in the bilirubin content of the splenic artery and vein during the hemolytic jaundice produced by injections of distilled water. Indeed, not even all cases of pernicious anemia show a higher bilirubin content in the splenic vein and it may or may not be found in secondary anemias (103), (83). These facts make it clear that the conditions under which the participation of the spleen in bile pigment formation can be demonstrated are variable and not clearly understood, and that there is at present no evidence that this organ plays a major or dominating rôle in this connection normally. However, it is entirely possible that bile pigment may be continually formed in the spleen under normal conditions and discharged from it in amounts too small to be detected by our present methods. One recalls that, in the dog, no bile pigment can be detected in the blood stream in any part of the body unless its excretion by the liver be interfered with long enough to allow it to accumulate in the blood to the level of detection by our present tests. But the fact that, in the dog, splenectomy does not appreciably influence the excretion of bile pigment from a bile fistula (Whipple and Hooper (115), Inlow (38)), not even in the presence of severe intravascular hemolysis (Rich (76)), speaks against any very

essential or specific importance of this organ in the normal formation of bile pigment.

What of the old problem of the rôle of the *liver* in normal bile pigment formation? Since bile pigment continues to be formed after removal of the liver, it seems reasonable to believe that normally at least a part of the daily output of bile pigment may be formed by this extra-hepatic mechanism, whatever it may be. But how great a part of the daily output? Mann, Bollman and Magath (58) state that bilirubin appears in the plasma of dogs quite as soon after hepatectomy as after ligation of the common bile duct. If one regarded the ligation of the bile duct and the removal of the liver as two entirely similar means of preventing the escape of the pigment from the plasma, one would have to conclude from these experiments that all of the pigment is formed outside the liver normally. But when the bile duct is tied it does not follow that from that moment bile pigment ceases to be removed from the circulation by the liver cells. On the contrary, it is to be expected that the liver cells will continue to remove the pigment from the plasma and to pour it into the canaliculi and ducts until these are distended to the point of hindering further excretion into them. That this is actually the case is borne out by the studies of Bloom (10), in this laboratory, who has shown that at the time of the first appearance of a detectable amount of bilirubin in the blood stream of a dog after ligation of the common duct and removal of the gall bladder, not only the larger bile ducts but the intercellular bile canaliculi throughout the liver are distinctly distended with bile. It is clear, then, that following ligation of the common duct a very considerable quantity of bile pigment is formed and thrown into the bile channels before there is any retention of the pigment in the blood. Thus even if the pigment appears in the plasma as soon after hepatectomy as after duct-ligation one cannot assume that the same amount of it was formed under both conditions.

In thirteen experiments (lasting from one to five and a half hours and averaging over two hours) no bile pigment appeared in the plasma of dogs when hemoglobin was introduced into the blood stream circulating through the head and thorax only; but, on the other hand, if the liver alone were added to the head-thorax circulation by allowing blood to pass through it by way of the hepatic artery, bile pigment appeared regularly in the plasma even as early as one hour after the introduction of the hemoglobin and ligation of the common duct (79). For numerous reasons the writer does not regard it as proper to consider such a procedure as the examination of the plasma for bilirubin after

duct-ligation as an accurate method for estimating the amount of bile pigment formed in a given time; but it seems evident that if any conclusion is to be drawn from this method it should be that much more bile pigment is formed when blood passes through the liver than when it does not. And, indeed, Mann, Bollman and Magath state that in their experiments the rate of formation of bilirubin after hepatectomy was less than the rate of excretion of the pigment from the normal liver. Makino (56), making use of Mann's method of hepatectomy, gives figures from his experiments which indicate that about one-fifth of the normal daily amount of bile pigment could be formed in the absence of the liver. The marked decrease in the formation of bile pigment which is said to follow the reduction of the blood supply to the liver attendant upon the Eck-fistula would be further evidence of the importance of this organ in normal bile pigment formation. This is, however, a matter which deserves more careful study, for the actual extent or nature of the disturbance of bilirubin formation produced by the Eck-fistula is by no means clear. Whipple and Hooper (119), Matthews (60), Voegtlin and Bernheim (106), Pearce, Krumbhaar and Frazier (73) and others agree that a dog with an Eck-fistula forms considerably less bile pigment than a normal dog, but they differ widely in the degree to which they find the production of the pigment disturbed; and while Whipple and Hooper state that bilirubin is formed from injected hemoglobin as rapidly by Eck-fistula dogs as by normals, they submit a certain amount of evidence that the Eck-fistula dogs convert less of the injected hemoglobin into bile pigment than do normals (117). There seems to be no doubt that in birds the liver is by far the most important organ in bile pigment formation. It is entirely possible that in mammals this organ plays a similarly important rôle under normal conditions, and that it is only under pathological conditions that the extrahepatic mechanism is brought into active play. This view is plead for strongly by Eppinger (17), and we have certainly other examples of compensatory reactions in connection with hemoglobin metabolism—witness the well-known fact that although the building up of hemoglobin is a function of the bone-marrow in normal adult life, other tissues, especially the liver and spleen, can readily assume this function when the bone-marrow is damaged; and that in the animals in which phagocytosis of red cells and their fragments is confined chiefly to the spleen, after splenectomy the reticulo-endothelial cells of the liver, lymph glands and bone-marrow may become markedly active in erythrophagocytosis (33), (73). Indeed, if the

reticulo-endothelial cells should actually turn out to be the producers of bile pigment, the enormous numbers of these cells in the liver would straightway indicate its importance in this regard. At present, however, we are unable to speak with certainty concerning the exact rôle of the liver in normal bile pigment formation except in so far as we are sure that the *epithelial* liver cell is not necessary for the formation of the pigment.

We may summarize our information on the question of the formation of bile pigment by stating that *hemoglobin may be regarded as a source of bile pigment, and we have no proof that there are other sources. There is no proof that the epithelial liver cells can form bile pigment under any circumstances; on the other hand, it is certain that the pigment can be formed outside of and independently of the activity of the liver cells. There is evidence that cells of the reticulo-endothelial type can form bile pigment, but this evidence is not yet complete enough to be regarded as proof. There is no evidence whatever that any other type of cell can form the pigment, nor is there any proof that the pigment can be formed extracellularly by enzyme action. We are still unable to speak with certainty about the normal site of origin of bile pigment.*

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THE INFLUENCE OF MUSCLE WORK ON PROTEIN METABOLISM

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The problem of the metabolic phenomena which occur in the contraction of muscle is one of perennial interest. As the result of recent research, for instance that of Hill, Meyerhof, Embden and others, the carbohydrate aspect of the question at the moment looms large and there is perhaps a tendency to lose sight of the fact that the actual functional tissue involved is composed of material which is at least potentially combustible. The question may well be asked whether muscular work influences in any way the functional tissue.

The carbohydrate aspect is but one phase of the problem. It may indeed be the dominant phase, but it is certainly not the only one concerned in the genesis of the energy requisite for one of the most fundamental of physiological phenomena. It is probably true, for there is an immense amount of evidence in favour of it, that carbohydrate is the most rapidly available source of energy; but even on this point there is much evidence to show that the problem is not a simple one. For example, the study of the Respiratory Quotient during prolonged work at least does not support the view that carbohydrate is the sole substance utilised (1), (2).

The organism, so far as its active tissue is concerned, is built up of a complex substance, protoplasm, which certainly plays the vital part in the various metabolic processes. These processes consist not merely of the production of work, heat, etc., essentially catabolic phenomena, but also of the equally important anabolic phase associated with tissue restitution. Even if the carbohydrate supply be actually stored in the various cells in discrete form it must come into more intimate contact with the active metabolic tissue before it is utilised. Whether or not it ever becomes an integral part of the active cell tissue there is no indubitable evidence. The reading of modern work would suggest implicitly perhaps rather than explicitly that the material yielding the energy requisite for muscle contraction is a fuel which is, so to speak, stoked on to the cellular furnace, that, in other words, a superimposed temporary metabolism is possible.

The problem so far as protein is concerned may be considered in two ways; one indirect, the other direct. 1, Does muscular work increase the demand for protein and inferentially thus demonstrate the need for material to make good a loss; and 2, does muscular work lead to increased catabolism of the functional tissue and thus increase the output of nitrogen in the urine?

Many workers hold that valuable although indirect evidence, may be obtained of the whole organism's requirements and consequently of the true demands of active tissues from the study of people's and individual's dietaries. The protest may be made that deductions drawn from the dietary habits of generations of peoples in all quarters of the globe are fallacious, as the intervention of factors of unknown value or importance may have vitiated the observations. Long-established food customs cannot be ignored for, as Sir William Roberts well put it, "they are the fruit of a colossal experience accumulated by countless millions of men through successive generations." If one may then venture to attack the problem from this direction, it may well be asked, if carbohydrate is to be regarded as the sole or principal foodstuff intimately associated with the genesis of energy in the muscle why is it that the demand for an unlimited supply of starchy food during the performance of hard muscle work is not clamant? Those engaged in strenuous physical work do not crave for sugar and white bread; they want a good mixed diet in which carbohydrate is present but is not unduly prominent.

The great majority of the training systems, both modern and ancient, lay particular stress on protein, and most frequently on meat, as an article of diet. Further they all tend to reduce the intake of carbohydrate. Content of protein rather than caloric intake is the point unconsciously emphasised. The same is apparently true where, instead of training for a contest, regular hard muscular work has to be performed. Practically wherever one turns it is the same picture that is presented. Given free choice, the worker instinctively selects a relatively high protein intake. McCay's (3) work showed very definitely and conclusively that in India, with its teeming millions of diverse peoples, the strong virile fighting races are large consumers of protein, a conclusion in which he is supported by Campbell (4). In the infinitely more limited but carefully devised marching experiments of Melville, the importance of the supply of protein was also stressed. Perhaps Mulder (5), to whom we owe the term protein, was not so very far wrong when he wrote in 1847 "Der Löwe ist so gereizt, so kräftig,

nicht nur weil er als Löwe gebaut ist; sondern auch weil Löwenblut in seinem Adern strömt. Das Löwenblut stammt nicht von Reis oder Wurzeln her, sondern von Fleisch. Die Kuh ist so ruhig wie sie ist, nicht nur weil sie als Kuh gebaut ist, sondern auch weil ruhiges Gras- oder Heublut in ihren Adern fliest. . . .Deshalb, wer zuviel Kraft in sich spürt, er esse wie die Kuh Gemüse; wer zu wenig Kraft hat, er esse wie der Löwe Fleisch."

Incidentally it may be remarked that the third energy-giving food principle, fat, has never been the subject of such acute controversy. There is a great deal of evidence to show however that, where very hard muscular work is to be done, an increase in the fat content of the diet is craved; thus in the dietary of lumbermen and others engaged in hard work in the open air the fat content of the diet may account for some 40 per cent of the total calories. Lavonius (6), who studied very carefully the metabolism of two professional wrestlers, found that one ingested 218 grams protein, 260 grams fat and 431 grams carbohydrate per day and the other 182 grams protein, 205 grams fat and 392 gram carbohydrate. The percentage of calories from protein, fat and carbohydrate respectively was, thus, as follows, *1*, 17.6, 47.6 and 34.8 and *2*, 17.5, 44.8 and 37.7. It is evident then that both the hard-working lumbermen and these strenuous Finnish athletes resemble very closely in their intake of fat the Eskimo with his 48 per cent of calories from this source.

The value of fat as a fuel food was emphasised many years ago by Frankland (7). He records "I well remember being profoundly impressed with the dinners of the navigators employed in the construction of the Lancashire and Preston Railway: they consisted of thick slices of bread surmounted with massive blocks of bacon in which mere streaks of lean were visible. These labourers doubtless find that from fat bacon they obtain at the minimum cost the actual energy required for their arduous work." Fick and Wislicenus (8) in their classic paper put forward in support of their conclusions that "the chamois hunters of Western Switzerland are accustomed when starting on long and fatiguing expeditions to take with them as provision nothing but bacon fat and sugar because as they say these substances are more nourishing than meat." Lawes and Gilbert (9) also emphasised the fact that people with hard muscle work to do resort to meat rich in fat rather than to the lean varieties.

Krogh and Lindhard (2) have found no evidence, when the working subject was kept on a diet rich in fat, that "fat must be converted into

sugar before being utilised for muscular work." This result would seem to settle Chauveau's isoglycosic hypothesis.

Let it be granted then that the main supply of energy is drawn from non-nitrogenous sources still the substance of the muscle is rich in nitrogen. It would be ridiculous to argue as if muscle were a stable mechanical arrangement like a practically unwearable jewelled bearing. The fact that, if the conditions are not optimal, there is very soon unmistakable evidence of excessive wear and tear, is definite proof of the delicate chemical balance which exists in muscle. The question must then necessarily be raised, Is there no evidence of an increased output of nitrogen as the result of muscle activity?

As long ago as 1855 Beigl (10) wrote an excellent paper in which he dealt in a most interesting fashion with the output of N in the urine and and the factors which influence it. Amongst other conditions he tested the effect of work on the output of six subjects upon various diets and compared the work-days' output with that of days of complete rest. When the diet was very meagre, as well as when it was abundant and rich in protein, a rise in the output of N followed muscle work. As a matter of fact the rise on the abundant protein-rich diet was the more marked of the two. Hammond (11) also, with apparently an adequate diet, found a rise in the output of N commensurate with the amount of work done. Traube (12), who was one of the earliest workers to stress the importance of N-free foodstuffs, concluded on the other hand that there was no evidence of the breakdown of protein during muscle activity. He believed that, as the result of the work, there was an enhanced demand for protein for repair purposes. E. Smith (13) was one of the first to study really measured work. He carried out his experiments on four prisoners working on a treadmill. He calculated the work done was equivalent to about a 29-mile walk. He found a definite rise in the output of N either on the day of work or on the day following. C. Voit (14) concluded from two experiments, both with single work days, that work produced no marked change in the output. In a later series of experiments, on a man turning a braked wheel, Voit working with Pettenkofer (15) stated that work had little effect on the output of N.

The classic experiment, which was held finally to displace protein from the position of primacy to which it had been raised by Liebig, has in the hands of many subsequent workers been made to prove more than the authors Fick and Wislicenus (18) ever claimed, namely, that

protein could not possibly have yielded all the energy expended during the experiment. Fick and Wislicenus climbed the Faulhorn, 1956 metres above the lake of Brienz starting from Iseltwald village on the morning of August 30, 1865 (?). The climb lasted about six hours. The last protein meal was taken at midday, August 29; thereafter, until 7 p.m., August 30, the food was essentially non-protein. The urine was collected in four portions.

Night urine. 6 p.m., August 29, till 5:10 a.m. August 30.
Before climb started at 5:10 a.m. they had breakfast including wine and beer "which was taken in the quantity usual in foot tours."
Work urine. 5:10 a.m., August 30 till 1:20 p.m. August 30 (i.e., duration of climb).
Post-work urine. 1:20 p.m., August 30 till 7 p.m. August 30. Resting in hotel at top of Faulhorn.
Night urine. 7 p.m., August 30 till 5:30 a.m. August 31. A protein-rich meal was taken at 7 p.m. in the hotel.

Although certain analyses were done in the hotel the urines were all re-analysed on their return to Zurich.

N output in grams per hour

	FICK	WISLICENUS
Night urine	0.63	0.61
Work urine	0.41	0.39
Post-work urine	0.40	0.40
Night urine	0.45	0.51

They calculated that Fick used 22.09 grams protein and Wisliceuns 20.89 grams during the period of the climb and, if the post-work output be also included 38.28 (or 37.17) grams, and 37.00 grams, respectively. They showed therefore that the combustion of this amount of protein could not have accounted for the energy expended during the climb in raising the body weight of Fick 66 kilo. and of Wislicenus 76 kilo through 1956 metres. In spite of the almost ludicrous defects of the experiment by a modern metabolic standard and despite certain overstatements their main conclusion is well founded, that "the substances by the burning of which force is generated in the muscles are not the albuminous constituents of those tissues but non-nitrogenous substances either fats or carbohydrates."

Later Voit (16) in Hermann's Handbuch discusses all his experimental work. Although he contends that work has little or no influence on pro-

tein breakdown, it will be noted that in the starving experiments on his thin animal there was an increase of N output of 8 to 16 per cent as the result of work; and when food was taken and work done, an increase of 7 per cent. In the case of the experiments on the old fat starving dog, the rise was from 1 to 8 per cent. In the human experiments, when work was done with the subject starving, there was no rise on the day of work and no post days are recorded; in the experiments when the man was fed a slight fall occurred on the first day of work with a slight rise on the second day. Again there were no post days.

Frankland (7) discussed the Fick and Wislicenus experiments and, after having determined the calorie value of the material involved in a very beautiful series of tests, he recalculated their results. Although he reduced the amount of work supposed to have been done, he held that only about one-fifth of the energy expended could have come from the protein used. Matteucci (17), whilst admitting the accuracy of Frankland's criticism and conclusions, still believed there was a certain amount of protein utilised during muscle work.

Parkes (18) may be regarded as the first worker to carry out a real series of work experiments on modern lines. One set of experiments was done on an N-free diet and another on a mixed diet. He found in both that exercise produced a slight increase in the output of N. In a later set (19), again using two soldiers on a fixed diet containing 19.61 grams N for 16 days, he found a slight rise in N output as the result of exercise (marching 24 and 35 miles). In a still later set (20) he came to the general conclusion that "force necessary for great muscular work can be obtained by the muscles from fat and starch though changes in the nitrogenous constituents of the muscles also go on which have as one effect an increased though not excessive elimination of nitrogen after the cessation of work." He raised the question whether it might not be possible that some re-utilisation of the nitrogenous material catabolised took place. Hermann (21) also raised the question as to the changes which took place in muscle during work in a most interesting paper. He believed there were two types of anabolism and two types of catabolism and he came to the general conclusion that when the amount of work done was moderate and even where prolonged and heavy, if the work periods alternated with adequate periods of rest, there was no evidence of any increased N output; whereas if the work were both heavy and continuous, there was definite evidence of N wastage.

Haughton (22) using very defective methods, reported that he found no increase in N output as the result of 5 days' walking and climbing,

whereas Weigelin (23) comparing the output of N on walking days (2 hours hard) and on resting days came to the conclusion that the exercise was followed by a definite rise in the output of N, the rise being most pronounced in the post-work period.

Liebig (24) in his well-known address on the source of muscle power, withdrew somewhat, mainly, it would seem, as the results of the experiments of Parkes, from his earlier position in which he maintained the supremacy of protein. He admitted that there was no direct relation between muscular work and the production of urea, but nevertheless "the nitrogenous compounds eliminated during muscular exertion are ultimately excreted as urea and that this does not take place completely at the time the work is done but at a later period." Voit (24a) attacked this address vigorously and maintained that his experiments negatived Liebig's conclusions. He also defended the work of Fick and Wislicenus against the quite proper criticism of Liebig that it was possible the N would have excreted on the days following the climb. He further raised many objections to the experiments of Parkes which, as a matter of fact, were much more scientifically carried out than his own earlier work. Speck (25) who next dealt with the problem was of the opinion that under normal conditions the N-free materials were to be regarded as the source of muscle energy and that only under exceptional circumstances were the N-containing substances used. Schenk (26), too, concluded that it was questionable if work had any influence on the N output, although an examination of his protocols shows that a definite, but slight, increase in the N output took place as the result of work. Thus in experiment I the output of urea, average of 2 prework days, was 46.23 grams, average of 3 work days was 50.99 grams and for the 4 post-work days the output was 50.96, 52.26, 44.78 and 46.21 grams.

Pavy (27), in a valuable series of observations, studied the metabolism of professional pedestrians, who, in the course of their demonstrations, did experiment I 180 miles in 2 days, experiment II 263 miles in 3 days and in experiment III 450 miles in 6 days. The urine was all collected and carefully analysed. The N intake was also determined; the amount of food consumed was apparently ample. Pavy admitted, rather unwillingly, that the N output during the days of walking was higher than the rest days. Naturally the protein utilised could not account for the energy expended. Austin Flint, who had previously (28) carried out a series of observations on one of the professional pedestrians utilised by Pavy, found a definite rise in the N output as the result of hard work

(63.5 miles walking per diem for 5 days) and criticised vigorously (29) this paper by Pavy, particularly his attempt to explain away the observed increase in N output because it did not fit in with his (Pavy's) theories on the subject. Flint's discussion of the whole problem is well worth reading in its entirety; his trenchant criticism of the attempt to force forward physical laws and mechanical explanations in explanation of physiological activities is admirable. His general conclusion is "that excessive and prolonged muscular exercise may increase the waste or wear of certain of the constituents of the body to such a degree that this wear is not supported by food. Under these conditions there is an increased discharge of nitrogen particularly in the urine." He quite pertinently asks the question why the muscular system needs rest and recuperation after prolonged exertion if it be simply "the food which has its force liberated and not the muscular system which wears itself to the point of exhaustion." He also criticises the Fick and Wislicenus experiments holding that "viewed physiologically without reference to any particular theory of the origin of muscular power the experiments of Fick and Wislicenus seem to have no great value," and pointing out, quite rightly, that the fall in the output of N in the work and post-work periods, could result from the ingestion of N-free food.

Breitzcke (30) also carried out a series of work observations on convicts comparing the output of N with that which occurred on days of no work. The body weight of his subjects remained fairly constant but in 4 out of the 6 used the work did bring about a definite rise in the output of N. North (31) and Winter Blyth (32) both examined the urine of subjects engaged in walking. North found a definite increase in N output but Blyth did not confirm.

Oppenheim (33), on the other hand, came to the conclusion that although work might be followed by a rise in the N output, muscle activity *per se* is not the causal factor. He adopts a suggestion of Fränkel (34) that it was the concomitant dyspnea which was responsible for the rise in N output. Oppenheim found that a large amount of work done at a slow rate brought about but a slight rise in the output of N; the *next* day he did the same amount of work at a faster rate so as to produce dyspnea, and obtained an increase in the N output; and on the *following* day, he again did the same amount of work still faster, so as to induce marked dyspnea, and got a marked rise in the N output. If the rise in the output of N as a result of muscle activity is frequently very marked, even most marked, on the day or two following the

exercise, these experiments on successive working days are without value. He carried out also a couple of experiments with a day of rest intervening with similar results. It may be noted here that this hypothesis of Fränkel was subjected to a severe criticism from Voit (35), who held there was absolutely no support for the assertion and suggested that the increased protein catabolism in dyspnea was due to muscle activity "wie bei der ubrigen Muskelarbeit." Fränkel (36) in a later paper replied to Voit's attack and reaffirmed his conclusion.

Hirschfeld (37) recorded a rather unsatisfactory series of experiments in which the diet would seem to have been deficient. There was a rise in the output of N most marked on the third day of work. Argutinsky (38) carried out a well-designed piece of research on a regulated diet. In each of the 4 experiments there was a definite rise in the N output. As it was possible that the diet though sufficient for the subject on his non-working days, might be inadequate when exercise was taken, Argutinsky in experiment IV added on the day of exercise an extra 100 grams of sugar the calorie value of which he calculated to be equal to twice the heat equivalent of the work done. Yet a rise in N output still took place. Bleibtreu (39) checked Argutinsky's analyses and confirmed them. Krummacher (40), also, working in Pflüger's laboratory, did a similar series of experiments to those of Argutinsky and found a similar rise in the N output. The calorie value of the food was low. Hirschfeld (41) held that Argutinsky's results were due to a deficient dietary. He carried out a series of experiments on an ample diet but, unfortunately, he apparently made no attempt to get into a state of nitrogenous equilibrium before exercise started. Thus one finds experiment II an intake of 5.95 gram N in the food and an output of 11.30 and 7.41 grams N on the two pre-days immediately preceding the work. In only one of the three experiments recorded is there any definite evidence of a rise in N output. Munk (42) also held that Argutinsky's results were due to a deficient food intake. He maintained that even the extra sugar taken to cover the extra calorie expenditure did not suffice. Noël Paton (43) also attacked the experiments of Argutinsky and Krummacher on the deficient calorie intake. He carried out experiments on a student, using a diet of 3979 calories containing 16 grams N with rest days before and after exercise. He got a small but definite rise of N on the day of work and a very definite rise on the first and second post days. The total increased output for the day of work and the two succeeding rest days amounted to 3.274 grams N. Pflüger (44), in whose laboratory the experiments of Argutinsky

and Krummacher were done, in an extremely interesting and characteristically forceful paper, put forward his slogan "Keine Muskelarbeit ohne Eiweisszersetzung." The N-free constituents of the animal body he maintained were dead substances, only the protein is living and capable of meeting all the special demands of life. He admitted that often the rise in the output of N as the result of work was not very marked but he held it was constantly present.

Krummacher (45), in putting forward a new set of experiments, admitted that his own previous series and those of Argutinsky were carried out on a deficient dietary. In his new series two of his experiments were carried out on an ample diet experiment I, 38 calories, II 64 calories and III 72 calories per kilo body weight. He found that a definite rise in the output of N amounting to 23 and 22 per cent respectively in the first two experiments and a slight rise of 7 per cent in the third. In the first two experiments the rise in output was most noticeable on the two days following the work, whereas in the third, although the work done was actually greater, there was no marked after-effect. Naturally in all the experiments the increased catabolism of protein in no way covered the energy expended on the work done. It is interesting to note that in this paper Krummacher defended the Voit position that any breakdown of protein which took place was not due to *direct* action of the muscle work on the protein molecule but might be the indirect result of the increased combustion of the N-free material. Voit, he says "hat niemals die Betheiligung des Eiweisses an der Muskelarbeit geleugnet."

Frentzel (46) carried out a series of experiments on dogs. In the first, where the animal was fed on a fat protein diet, as the result of two days' work on the treadmill there was a definite, though small, rise in the N output. The average of 5 pre-days was 6.11 gram N. On the first work day it rose to 7.29 grams and fell to 6.06 grams on the second work day, to rise again to 6.21 grams N on the first post day. When similar experiments were done with the dog starving, the rise in N output, as the result of exercise, was greater. Thus the total N output for 4 days previous to work was 14.35 grams the output rose to 20.70 for 3 work days and first post day. Caspari (47) also reported a similar set of experiments on dogs exercised on a treadmill. The food was all carefully analysed. After a pre-period of 7 days, three days of successive work were done. A definite rise in N output of about 4 grams took place on the second work day although there was a fall the third day. This was however followed by a rise again on the day fol-

lowing the cessation of work. In a second experiment, with more carbohydrate and less fat in the diet, a similar rise in the N output was obtained. Evidence of retention of N was obtained as the work progressed.

Dunlop, Paton, Stockman and Macadam (48) carried out 3 experiments on the output of N after muscle work. The calorie intake varied but, on the whole, it may be regarded as adequate. There was at any rate no marked loss of body weight. The N intake was high. They came to the conclusion that hard exercise, walking, cycling, digging, led to an increase in the output of N, an increase which was most marked on the two post-work days. The increased output they found amounted to from 5.3 to 7.9 grams. Heinemann (49) experimented with a man using the Gärtner ergostat. No attempt was apparently made to fix the N intake or, indeed, the diet generally. Nevertheless on both a protein rich diet and on a carbohydrate rich one there is evidence of an increased N output, whereas with a fat-rich diet there is an apparent reduction.

Bornstein (50) investigated specially the possible influence of muscular work on the retention of protein. He referred to the work of Morpurgo (51) who had studied the well recognised hypertrophy of muscle which results from exercise and who had shewn that the increase of the muscle was not due to any increase in the number of the cross-striated fibres but to a filling out of already existent elements—an increase in the amount of sarcoplasm. Bornstein went on a fixed diet for 7 days on a fairly high protein intake and obtained a daily retention of over 0.5 gram of N. Then, whilst still on the same basal diet, he added 6.75 grams N in the form of casein and now obtained a daily retention of about 1.5 gram. During this second period he did 17,000 kgm. of work daily on an ergostat. There was a definite rise in the output of N on the fourth day of work but thereafter it fell and kept on the whole fairly constant. He calculated he had retained 799.5 grams "flesh" or by another mode of calculation 773 grams and found, *mirabile dictu*, that his body weight during the period had increased exactly 800 grams. Zuntz (52), who discussed the papers of Caspari(1c) and Bornstein (1c) pointed out that the rise in N output noted by Caspari and others during muscle work and the retention of N found by Bornstein brought about hy work is a special case of Pflüger's law of teleological mechanics (53), viz., "die Ursache jeden Bedurfnisses eines lebendigen Wesens zugleich die Ursache der Befriedigung des Bedurfnisses ist." The retention of N as the result of muscle work was confirmed by Loewy (54) who found however no evidence of any rise in output.

Atwater and Sherman (55) studied the N balance of 3 subjects, professional cyclists, engaged in a 6-day bicycle race. In all 3 subjects they found that the output of N very definitely exceeded the intake. The extent of the excess output would seem to bear some relation to the amount of work done, as it was greatest in subject M. who averaged nearly 335 miles per diem, less in subject A. who did about 304 miles, and least in subject P. whose average was nearly 288 miles per day. Zuntz and Schumburg (56), in their classic study of marching, carried out a certain number of observations on the influence of this type of work on the N exchange. They found that marching brought about a well-marked rise in the output of N. The rise was observed not only in the post-work periods but also on the days when the work was done. The increase on the working days averaged, in the two sets of experiments, 2.4 and 2.6, on the post days 3.3 and 3.3 grams per diem. Although Kaup (57) concluded that no rise in the N output resulted from work a study of his data shows that, in his third experiment at least, a very definite rise in the output of N occurred. The average output for the two preceding rest days was 21.44 grams N; for the day of work 22.21 grams; the average output for the 3 post-work days was 23.38 grams, an increase of 0.77 gram on the day of work and for the three post days of 1.81, 2.66 and 1.35 grams or a total increased output over the basal pre-work period of 6.59 grams N. Frank and Gebhard (58) carried out some experiments on a dog whose activity had been reduced by the administration of curare, and found a *reduction* in the output of N. They found difficulty in reconciling this result with Voit's dictum that N output was not influenced by muscle activity, and suggested that the result was due not to reduced muscle activity but to failure to excrete the N for some reason unexplained. Voit (35) again dealt with the problem and pointed out that although muscle work had no direct influence on the degree of protein catabolism, yet a rise in the N output with work might take place. His general conclusion was that if N-free food was available in sufficient amount the effect of work on the output was slight.

Shaffer (60) experimented with two human subjects on defined diets. In those on subject O. T. he had four periods, 1, almost continuous rest, 2300 calories; 2, ordinary laboratory life, 3000 calories; 3, extra exercise, mainly walking, not excessive, 3200 calories; 4, ordinary life, free mixed diet N-rich purin free. On the fourth day of the exercise period there was a rise in N output over 1 gram, the exercise on the day previous having been most severe. The outputs of total N for the

4 days of this exercise period were 3.78, 3.96, 3.43 and 4.58 grams. The daily intake was 5.9 grams therefore although a rise in output took place it did not exceed the intake. The body weight of the subject increased from start to finish. In the second series of experiments on H. the periods of the different types of life alternated, but, if the exercise periods be averaged, there is some evidence of a slight increase in the N output over the other periods. This N output never exceeded the N intake. Thomas (61) whose subject worked on the ergostat whilst on a N free diet found only a minute rise in N output as the result of the exercise. Kocher (62) on the other hand also used a N free diet (1.01 grams N) with a calorie value of 5089 calories. The average output of N on the last two pre-days was 2.93 grams, on the work day (60 kilometre walk) 3.89 grams, and on the two post days 4.69 and 4.29, a definite rise. The experiment was repeated on another subject with a like, but not so striking, result, viz., 3.07 : 3.77 : 3.71 and 2.89. In another series on a very high N intake and 5412 calories subject I showed no great rise in the N output but in this instance no post-day values are given. In a fourth series with a somewhat lower but still high intake of N nearly 34 grams, and a calorie value of 3585, which, the author states, was not sufficient for subject I, there was a very definite rise in the N output after work. In a fifth series on subject II with an N intake of over 21 grams and a calorie value of 2186, the subject walked 35 kilometers at 6 km. an hour and instead of a rise in N output there was evidently some retention. Eckert (63) in the course of an investigation on protein metabolism came to the conclusion that the rise in N output which results from work indicates a definite breakdown of body protein. Atkinson (59) came to the opposite conclusion, working with a dog after the ingestion of a large quantity of meat. He could find no evidence that either the absorption or the hourly metabolism of protein was influenced by muscle work. Campbell and Webster (64), (65) carried out 4 five-day experiments: 1, complete rest in bed; 2, six and a half hours ordinary laboratory routine; 3, one and a half hours ordinary laboratory routine plus 5 hours work at 67500 kgm. per diem; and 4, 5 hours severe work at 95000 kgm. per diem. Unfortunately, although the diet is stated to have been controlled, no analysis of the exact N intake was made. A slightly increased output of N was found in the first work experiment and a definite rise as the result of the severe work. In this latter experiment, the N output rose from 8 grams on the first day (the output on the same diet when at complete rest in bed was 8.01 grams) to 10 grams on the

second day, 12 grams on the third day and then fell again to 10 grams on the fourth and fifth days. As administration of oxygen had no influence on the N output the authors conclude that defective oxidation cannot be the cause of the increased N output. They inclined to the belief that it was due to damage to protoplasm, both nervous and muscular, due to excessive fatigue and strain.

Working with Mr. Burnett I have recently concluded (unpublished) a long series, 14 in all, of work experiments on strictly controlled and analysed diets. We found that, although the subject's body weight rose throughout the duration of the experiments, which extended over more than twelve months, from 78.5 to 80.2 kilo, and he was in perfect training, there was a definite rise in the output of N as the result of the performance of a moderate amount of work. The work amounted to 25,000 kgm. performed in one hour and carried out on 6 successive days. The extent of the rise varied on different diets. In each of the experiments the average of the last four days of the pre-work period, the average of the six days of work and the average of the first four days of the post-work period were taken. *I* is the average output of N for 3 repeat experiments on a diet consisting of wholemeal bread, margarine, sugar and marmalade. *II*, the average output of 3 repeat experiments with a diet which consisted of wholemeal bread, cheese, margarine, sugar and marmalade. *III* is the average of 3 repeat experiments on a diet consisting of wholemeal bread, cheese, margarine, sugar, marmalade and meat extract. *IV* is the average output of 2 experiments with a diet of wholemeal bread, lean beef, margarine, sugar and marmalade. An apple or an orange was taken each day. The calorie value of each diet was kept at approximately 3000 calories.

Total nitrogen output in grams

	I 3 EXPERIMENTS	II 3 EXPERIMENTS	III 3 EXPERIMENTS	IV 3 EXPERIMENTS
Pre-work (average 4 days).....	8.78	17.31	17.40	14.30
Work (average 6 days).....	10.01	19.96	18.23	15.44
Post-work (average 4 days).....	9.05	17.76	17.34	16.34

The work was well within the powers of the subject, although the rate at which it was done on the arm lever ergometer, in order to perform the desired 25,000 kgm., would be found exhausting by most subjects. His respiration was never disturbed and sweating not excessive.

A large number of observations on the output of other excretory substances in the urine and on his respiratory metabolism were made.

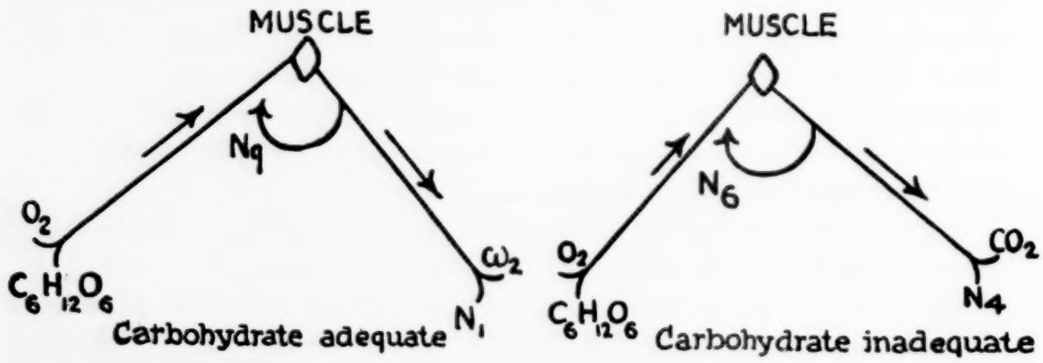
Despite the very definite conclusion of Professor Lusk (66) that it is fully proved that "muscular work does not increase protein metabolism" the accumulated evidence seems to me to point in no unmistakable fashion to the opposite conclusion, that muscle activity does increase, if often only in small degree, the metabolism of protein. Although there is no possible ground for the view that protein is *the* source of muscle energy it is really astonishing that the evidence points to a protein catabolism in active muscle of such small magnitude. The question must be raised, why is the outward and visible sign of wear and tear so slight as under ordinary conditions it is? We know however that muscle is a metabolically active tissue, built up of living cells, filled with highly reactive contents, where anabolism and catabolism are in constant operation. During muscle activity, within these complex living cells, material is somehow transformed so as to liberate the energy requisite for contraction. Associated with the liberation of energy there is a formation of acid. Meyerhof (67), Milroy (68) and others have definitely stated that the proteins of muscle must be assumed to play a rôle in the acid buffering phenomenon. It is known that changes in the hydrogen ion concentration of the tissue fluids do influence protein metabolism, and we have no reason to assume that, however perfect the buffering arrangements in muscle, the liberation of acid can be without influence on the cell contents. If protein combines with acid one might assume that the protein so utilised will be incapacitated for the performance of a part of its normal function for while buffering may well be one of the normal functions it certainly is not the whole function of protein.

A good deal of evidence exists to show that changes actually do take place in the muscle as the result of activity: to name a few only the changes in the purine content noted by Burian (69) and Macleod (70), in the creatine creatinine content noted by Brown and Cathcart (71) Pekelharing and vanHoogenhuyze (72) and by Uyeno and Mitsuda (73). Confirmatory evidence may be deduced also from the work, among others, of Pugliese (74), who examined the N content of 1, resting and tetanised dog muscle and 2, the arterial and venous blood of the same muscles. He noted both in arterial and venous blood at the end of tetanus a slight but definite increase in the non-protein nitrogen. Rakestraw (75) also examined the blood before and after short and long

spells of exercise and he too found a marked increase in the non-protein nitrogen. A very similar result was noted by Feigl (76) who found that 70 per cent of the men engaged in an army march showed a marked increase of the "rest nitrogen" of the blood. Schultz (77) found a definite rise in the output of creatinine as the result of activity.

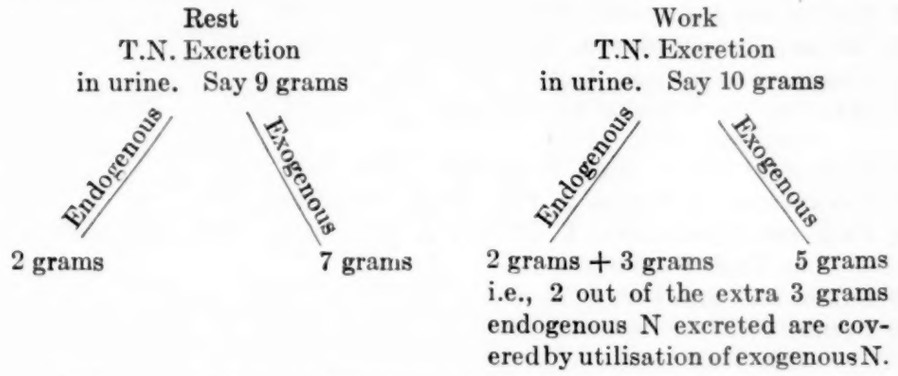
If one then assumes (and the whole problem abounds in unknown factors and assumptions) that in the normal course of events there is a regular throwing out of action of part of the protein element and a subsequent catabolism of this immobilised material, why is the output of N so small?

There are two possible explanations of this anomaly. The first is that after the disintegration of the protein the component parts may be for the most part utilised in resynthesis. The instability of cell protoplasm is illustrated by the fact that whenever there is a shortage of readily combustible fuel in contradistinction to the combustible but generally conserved protein basic structure, there is a very sharp rise in the output of nitrogen. It is possible, then, that given the optimum conditions, i.e., work done when the supply of carbohydrate both for the yielding of energy and for repair purposes is ample, the actual disintegration of protein is largely masked by the resynthesis which takes place. That any rise at all occurs in the output of nitrogen may be due to the flushing action of the greatly increased blood flow through active muscle.



The second possibility is the small output is due to the fact that while the real enhanced output, which would result from the complete excretion of all the nitrogen of the protein disintegrated in the course of muscle activity, actually takes place, the true state of affairs is masked by the utilisation of nitrogen-containing material, say of exogenous origin, which in the natural course of events would have been catabo-

lised and excreted, but which, under the special conditions, is deflected, taken up and retained in the muscle to make good the activity loss. Thus if we call the muscle-protein nitrogen, endogenous N and the food-nitrogen, exogenous N, the following schema may assist in making the proposition clear. (It must be understood however that although the exogenous N may, and probably does, arise from other sources than recently ingested food, for purposes of simplification of the explanation we shall adhere to the fiction that it comes from exogenous food sources alone.)



Dietary and other conditions apart from muscle activity constant.

It may be said that such an explanation is well nigh incapable of support, far less of proof, but such is not the case. There is certainly one very definite piece of evidence in its support, viz., the work of Bornstein and others, who have studied the special hypertrophy of muscle with the associated retention of nitrogen which takes place when muscle work is done. It is a familiar fact that well-graded and steady muscle work leads to the development (hypertrophy or eutrophy) of muscle. During the performance of muscle work then there is a retention of protein.

The problem is one of great complexity as concurrent with this stimulation of metabolism there are functional modifications, physical and chemical, taking place in and around the active muscle cells, and these changes probably have their own special effects in the direction of increasing the output of nitrogen. Of these modifications the most important in this connection seem to me to be the greatly increased blood supply and the increased mobilisation of water (it is of course well known that the water content of muscle increases during activity). The conclusion then is there is abundant evidence in support of the view that muscle work leads to a definite though perhaps limited increase

in the output of nitrogen, i.e., to increased catabolism of protein and at the same time there is also good evidence that the same muscle activity leads to the laying down of protein, i.e., to the stimulation of the anabolic processes in the broadest sense of the term. In other words the existent evidence points to a definitely enhanced metabolism of protein as the result of muscle activity.

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THE INHERITANCE OF ACQUIRED CHARACTERS

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INTRODUCTION. The idea that a bodily modification acquired during the lifetime of the parents can be transmitted to the offspring is at once both ancient and persistent. That the idea is ancient is shown by the prophecy of Jeremiah (about 600 B.C.)—"In those days they shall say no more the fathers have eaten sour grapes and the children's teeth are set on edge." Nevertheless, in spite of the major prophet, it has persisted; for Bernard Shaw recently delivered a typical Shavian invective in his *Back to Methuselah* in which he excoriated those who refuse to believe in such inheritance. Observation and experiment have given overwhelming evidence that organisms respond to changes in their environment or to changes in their usual functional activities, and that such responses are frequently of an adaptive type. The question we are considering is whether the effects of such responses reappear in part or in toto in the progeny after the original stimulus has been removed. It should be stated at the outset that the subject is still a matter of bitter controversy with, perhaps, the weight of opinion at the present time on the negative side; i.e., such bodily modifications are not transmitted.

Much has been written in defense of conflicting views, and the question has seemed to be as interminable as vitalism versus mechanism, or free-will versus determinism. The adherents of either view have frequently been fully convinced of the correctness of their position and therefore discussions have often been characterized by a spirit of decided partisanship. Competent biologists have been arrayed on both sides; and, fortunately or unfortunately, whichever view we take, we find ourselves in respectable company. It will therefore be no easy task to give a *fair* estimate of these conflicting views in the light of such recent biological data as we can conveniently compress into a brief paper. Obviously, time and space preclude an exhaustive analysis of all supposedly pertinent data. The whole question has more than a purely academic interest. The relatively permanent effects of continued effort and training by ascendants on descendants, the possibility

of transmitting an acquired immunity and similar questions engage the attention of educators, physicians and sociologists. In a broad sense, however, the question is primarily one of evolutionary theory.

The fact that living creatures have evolved is perfectly clear and indubitable to almost every student competent to pass judgment on such questions, but the modus operandi of evolution has not been equally clear, we must frankly confess. No reasonably convincing explanations attracted general attention until Lamarck and Darwin framed their respective theories. Lamarck concluded among other things that variation and therefore evolution was the direct result of environmental agencies, or the result of use and disuse of anatomical parts and physiological functions. Without demonstrating it he assumed the inheritance of such changes. His theory has been rejected by many as unsatisfactory since the inheritance of such acquired bodily modifications is usually thought to be impossible. Darwin's theory rested upon four sets of facts,—the extremely rapid increase in numbers of living forms, variation, natural selection, and heredity. In the struggle for existence following competition with overwhelming numbers of living organisms or following the exigencies of a changing environment those variates, which happened to be well adapted, survived, and therefore had opportunity to transmit their adaptive characters to subsequent generations. While Lamarck had tried to explain adaptive variation, Darwin merely assumed variation as a general inherent characteristic or quality of all living organisms. From Darwin's viewpoint, adaptation was as a rule the residual condition left after natural selection had eliminated the poorly adapted. But curiously enough we also find Darwin sometimes resorting to the Lamarckian explanation of individual or ontogenetic adaptations leading to racial or phylogenetic characters.

As early as 1875, Sir Francis Galton stated that the theory of the transmission of acquired characters included "much questionable evidence usually difficult of verification." The leading antagonist, August Weismann (1893), (1902), (1904) challenged the possibility of transmitting a bodily modification, in part because much of the evidence was of a superficial and anecdotal sort, and in part because he could neither find nor conceive of any mechanism whereby such transmission was possible. The former argument is pertinent; but the latter, as it stands, is an argument from ignorance, and one can never be sure that such a mechanism is impossible and may not be discovered. The cardinal features of Weismann's hypothesis are the relative isolation and inde-

pendence of the germ-plasm and its continuity from generation to generation. It is isolated and independent in the sense that it is set apart and is not in general contact with the somatic tissues and the stimuli which play upon them (at least in the higher animals, although in the protozoa and in plants this contention is certainly untenable). It is continuous in the sense that it is supposed to be derived intact from germ-plasm of the preceding generation, without elaborate differentiation and specialization like many somatic cells. The physical structure of the egg and spermatozoön determine the potential inheritance of the fertilized egg and the resulting body; but the body in turn does not determine what the structure of its germ-plasm may be. Any circumstance or play of the environment which alters the body does not of itself modify the germ-plasm in a corresponding way simply because it modifies the body. Removing, mutilating or changing cells or parts of the body do not produce correlated changes in the germ-plasm which induce similar phenomena to appear in the offspring. And so the Weismannian declares evolution is determined by changes in the germ-plasm itself but these are fortuitous, and perhaps just as likely to appear in one direction as another. The neo-Darwinian of to-day is a pure selectionist and looks upon evolution and racial adaptations as the result of natural selection which perpetuates only those individuals whose germ-plasm varies in a direction producing adaptive characteristics. That which is left after the process of elimination gives the appearance of adaptation, but emphatically without any teleological implication (unless there be perhaps a larger teleology which embraces purpose in the entire order of the universe). It must be admitted at the outset that the great majority of biological experiments and studies of the last fifty years rather favor Weismann's position. The proponents of Lamarckian principles have practically disappeared, with the exception of a few paleontologists and students of geographical distribution, and occasionally a pathologist or psychologist.

Historically, Lamarck's theories have passed through vicissitudes. The reception was at first luke-warm, but a revival of interest was stimulated by Spencer, Cope, Packard and others. As a result of the debates, Weismann and the neo-Darwinists in general have held the field almost undisputed. While the question has always been one of unfailing interest, recent observations and investigations (together with a growing skepticism of the complete sufficiency of natural selection to explain adaptive evolution and specialization) have led to a reawakening of interest in this subject.

In much of the discussion concerning the inheritance of acquired characters[1] there have been misunderstandings due to ambiguity of terms. It would be generally conceded at the present time that such inheritance of acquired characters means *1*, the production of a definitely *new* somatic character by a change in environment or functional activity; and *2*, the new character must reappear in the offspring at least in part after the removal of the original stimulus. The evidence bearing on this question is derived from two main sources:

A. A great body of suggestive or circumstantial evidence drawn from the appearances of certain natural phenomena, especially in the fields of embryology, paleontology and ecology.

B. The results of direct experimental tests and observations.

A. THE SUGGESTIVE OR CIRCUMSTANTIAL EVIDENCE. There are numerous examples of individual adaptation, as everyone knows. Illustrations can be given almost without end. Definite adaptive modifications both anatomical and physiological follow as responses to definite stimuli. Thus increased friction or pressure leads to callosities or epidermal pads; increased light to increased pigmentation; increased muscular or glandular exercise to increased size, strength and capacity for work; changes in temperature and humidity to acclimatization; unusual poisons and toxins to tolerance and immunity; and the like. But are these individual adaptive responses ever worked over into racial adaptations or hereditary characters, when the same stimulus is followed by the same response over a span of many many generations; and, if so, can we find out how it is accomplished? Certain lines of evidence from embyrology and paleontology have suggested to some biologists that something of this kind does take place. Thus Darwin and others have seen in the thickened epidermis on the soles of the feet of the human embryo the inheritance of an acquired bodily modification; for, said Darwin (1883, p. 33) "in infants long before birth, the skin on the soles of the feet is thicker than on any other part of the body; and it can hardly be doubted that this is due to the inherited effects of pressure during a long series of generations." A similar case was recorded by Thomson (1913, p. 180). "The African Wart-hog (Phacochoerus) has a peculiar habit of kneeling down on its fore-limbs as it routs with its huge tusks in the ground and pushes itself forward with its hind-limbs. It has strong horny callosities protecting the surfaces on which it kneels,

[1] It is perfectly obvious that every new character is in a sense an acquired character. The expression "inheritance of an acquired character" clearly means the inheritance of an acquired somatic modification.

and these are seen even in the embryos. This seems to some naturalists to be a satisfactory proof of the inheritance of an acquired character. It is to others simply an instance of an adaptive peculiarity of germinal origin wrought out by natural selection." This case was originally described by Leche (1902).

In studying the evidence for and against the inheritance of acquired somatic modifications, we soon learn that alternative explanations are often possible. This has led to endless confusion. None of the cases of evidence is as simple and convincing as it appears at a first and cursory examination. Relative to these particular cases we must credit Walter (1913) with a very pertinent suggestion. He observed that even the "mud-puppy" (Necturus maculatus) showed a much thickened sole. Now, Necturus is a very primitive type always living under water and never bringing any great pressure or weight to bear on the soles of its feet. "Nor" says Walter, "is it *reasonable* to suppose that it ever had any ancestor who did so for the hands and feet of the Amphibia are the most primitive and ancient hands and feet to be found in the animal kingdom without any known ancestral types. The thickening of the skin on the sole of the mud-puppies' feet *must* be due, *therefore*, to germinal determiners and is in no way an acquisition through use. The same may also be true of the wart-hog's knees and of human soles." (The italics are mine.) I suppose there is no field of biology in which we are (the writer includes himself) more prone to commit logical fallacies than in this field, and we continually find interesting examples of begging the question or reasoning in a circle in both Weismannian and Lamarckian arguments. Both have often taken recourse to well-known methods in argument in which the conclusion seems to be more firmly established than it really is because questionable premises are cleverly worded and taken for granted. For example, with the Weismannian we may state it *unreasonable*[2] to suppose Necturus ever had ancestors which acquired these skin thickenings through use and *therefore* they *must be* due to germinal determiners whose origin is unrelated to ancestral experience. But is it really proven that the ancestors of Necturus never habitually applied pressure or friction to the soles of their feet? Or do we know that the small

[2] Of course, no one wishes to be unreasonable,—fallacy of Petitio Principii, or question-begging epithet in which we practically assume the point at issue by clever wording and thus create a prejudice in the argument. It is a rhetorical device in which we all indulge at times and is especially common in discussions on the inheritance of acquired characters.

amount of friction which Necturus develops as it crawls along the bottom or behind rocks and stones, would not develop some skin thickening? No doubt the thickened sole and the horny-knee under discussion are due to germinal determiners and neither neo-Lamarckian nor Weismannian would deny this; but, it seems to me, the real question is, how did these appropriate germinal determiners get into the germplasm. The Lamarckian says "See how the habit led to appropriate germinal determiners for the necessary racial character" and the Weismannian replies "Behold, a germinal change determined the structure which made the habit possible." Regardless of whether the habit preceded the hereditary structural character or vice versa, any *new hereditary* structure has its origin in chance germinal variations subjected to the action of natural selection, according to the Weismannian. As a *formal explanation*, it is convenient, but not necessarily acceptable as convincing and final.

In a recent very suggestive and lucid paper Duerden (1920) shows that sternal, pubic, alar and median mesotarsal callosities are already well defined in the unhatched ostrich. Now curiously enough the first three callosities occur exactly in those regions where they would develop through the daily habits of the ostrich. Duerden also suggests that the last callosity (mesotarsal), although unused at present, may be related to the ancestral three-toed condition, in which condition the ostrich would have rested squarely upon its ankle. With the loss of the inner or second toe the whole tarsus would tend to turn inwardly and the median callosity would become unnecessary but has persisted up to the present time. Duerden shows that a new lateral-mesotarsal pad is not represented in the embryo, but develops when the chicks are a month or two old; for instead of resting its weight when in a crouching position squarely on the hereditary mesotarsal callosity, it supports itself on the inside of the ankle, thus giving rise to an adaptive somatic character, the new lateral-mesotarsal callosity. This callosity is reacquired during each ontogeny but has not yet become a phylogenetic character,—and we do not *know* that it ever will become such. But it at least proves the responsiveness of the skin of the ostrich to pressure, and that the individual response is adequate to meet the individual needs. Therefore, if there is inherent power to form effective epidermal thickenings during the course of ontogeny and in response to the individual needs, we may at least legitimately wonder why it is necessary to have these anticipatory, hereditary callosities appear in the embryo before there is any demand made upon the organism, as in the case of

the ostrich, the wart-hog and man. Why do they not recur in each lifetime entirely through individual adaptation as indeed it appears they can?[3] We may say with a formal selectionist explanation that there were fortuitous variations in the germ-plasm, which determined callosities at exactly those places where future needs would demand them. But is it not extremely improbable that chance variations in the germ-plasm would arise to determine such callosities at exactly those points? Why not fortuitous germinal variations for callosities elsewhere or almost anywhere on the body,—which should persist for they would have little or no lethal effect in the process of natural selection? In this remarkable case described by Duerden, as in many other similar cases we always come back to a dilemma. From the neo-Lamarckian viewpoint, the habit developed callosities as individual adaptations; and these were eventually established as germinal or racial characters, there being a direct causal relation between the ontogenetic and the phylogenetic character. The repeated acquisition of callosities in each generation eventually produced the racial character; why and how we do not know. From the neo-Darwinian viewpoint, the ostrich might acquire somatic callosities during ever so many generations, but this would bear no relation to the racial or inherited callosity, the latter being due to a germinal mutation in the "right" or at least in a fortunate direction. Indeed there may have been germinal changes in several directions, but in that event natural selection would be invoked to explain the survival of the more adaptive type of callosity. Which appeared first, the inherited characters that made it possible for the ostrich to develop its habit, or the habit which perforce compelled each ostrich to develop callous pads during its ontogeny until some fortunate coincidental change in the germ-plasm arose (entirely unrelated to be sure to either the habit or the ontogenetic callosities) and determined callosities at exactly those positions where they would probably have developed as individual adaptations in any event.

After all, what advantage in natural selection would an inherited callosity as a racial character have over an equally effective ontogenetic one? Indeed, some biologists fear natural selection alone makes violent demands on our credulity in some cases. The element of cogency cannot be neglected in our explanations. In any event, we wonder whether the habit, the ontogenetic callosities, and the germinal changes

[3] The writer does not mean to commit himself by asking these semi-rhetorical questions. The purpose is simply to point out the type of argument with which we are repeatedly confronted.

could not have developed *pari passu*, with some causal relationship between them, even though we do not understand just how it was accomplished. We may justly fear we are making a formal and universal selectionist explanation too easy, naïve and simple when we refer such hereditary adaptations to "spontaneous" chance variations in the germ-plasm, followed by natural selection.

Other similar cases lead to the same sort of dilemma from which there is at present no completely satisfactory escape. Whether we speak of the blind fish or amphibians of dark caves, or of the asymmetrical loss of pigment in the flounder, or of the instinctive habits of birds, insects and mammals or even of pigment-variations in the human race we are usually left in a state of doubt. The neo-Lamarckian explanation does not explain *how* the appropriate determiners for a purely somatic character could become incorporated in the germ-plasm and thus give rise to a racial character. The neo-Darwinian explanation does not explain the occurrence of any germinal variation; much less the fortunate concatenation of several, perhaps many, correlated germinal changes in the right direction, at the right time, and of sufficient magnitude to have any survival-value in selection. Kellogg (1907) aptly stated, "When species differences and adaptations are identical with differences and modifications readily producible in the individual by varying environment, are we not justified, on the basis of logical deduction to assume the transmutation of ontogenetic acquirements into phyletic acquirements, even though we are as yet ignorant of the physico-chemical or vital mechanism capable of effecting the carrying over? Has natural selection's claimed capacity to effect species change, unseen by observer, untested by experimenter, any better or even other proof of actuality than that just offered on behalf of species modification as a direct result of the stimulus of varying environment and functional exercise? I cannot see that it has."

The illustrative cases just cited are perhaps more difficult to understand and explain convincingly on Weismann's hypothesis than many cases are. The wingless beetles of the Madeira Islands may perhaps owe their origin to simple germinal variations in that direction; for the winged forms were blown out to sea, and the wingless survived,—although it should be added that Darwin (1883) did not hesitate to say that the action of natural selection may have been combined with the hereditary effects of disuse in this case. Similarly the gradually increased length of the trunk in the ancestors of the Proboscidea may have been due to a series of germinal variations. After the variation

had once taken place, the animal was "preadapted" to take up new habits. The ground parrot of New Zealand (Nestor notabilis) was originally insectivorous and fructivorous but with the introduction of sheep it suddenly became a predaceous carnivorous pest, tearing into the backs of sheep to get the deep layers of fat. Referring to this case Conklin (1921) says, "it was evidently well fitted or 'preadapted' for this new kind of food even before the food appeared. All such fitnesses were developed without regard to their later use; they are therefore preadaptations." In other words, some changes of structure may have preceded some changes of function or habit, but it does not follow that all cases are alike in this respect.

Those astonishing and freakish plays of evolution seen in cases of mimicry or protective resemblance are often difficult to explain on either a Lamarckian or Weismannian basis. Referring to Kallima, the dead-leaf butterfly of the Asiatic tropics, two competent biologists differed as to the part natural selection may have played. For one it proved too much, since the elaborate over-refinement in mimicry seemed absolutely unnecessary and it was difficult to conceive why the details of mimicry had to be carried so far by a process of natural selection. For the other, it simply proved that natural selection acting on germinal variations *could* produce mimics whose protective color and form involved even the most elaborate details. However neither writer saw any necessity of invoking a Lamarckian explanation, for such cases do not lend themselves readily and plausibly to this type of explanation.

While the Weismannian hypothesis was receiving a general cordial recognition, the paleontologists were not among those most easily convinced. The groups of facts and observations with which they were dealing did not lead to an unqualified acceptance of neo-Darwinism, and therefore they 1, either clung more or less tacitly to neo-Lamarckism with a feeling that its validity would be proven sooner or later; or 2, they turned to orthogenesis in which variation was predetermined in direct lines; or 3, they insisted on an open mind in the search for factors in evolution, as yet unknown. As Osborn (1895) stated, thirty years ago, "If acquired variations are transmitted there must be, therefore, some unknown principle in heredity; if they are not transmitted, there must be some unknown factor in evolution." The truth of the epigram is just as pointed to-day. No student denies the efficacy of natural selection; for both orthgenetic variation (as in the case of the coiled Ammonites, and as in the giant antlers of the Irish stag) and hypothetical Lamarckian phylogenetic adaptations must all sooner or later

in changing environments come under the influence of the final censor, natural selection, which decides whether a variation shall persist as useful or even as indifferent and harmless, or whether it shall be eliminated as disadvantageous and unfit.

As the case stands to-day, the neo-Darwinian or Weismannian point of view has decided advantages as a plausible formal theory for the development of many characters with definite survival-value. It meets with certain logical difficulties when applied to the degeneration of useless or superfluous parts. We have been using Weismann's hypothesis so long as a valuable pedagogic adjunct in giving to the sophomore a clear picture of inheritance as a definite process, that we have frequently overlooked, neglected or forgotten its shortcomings. Weismann himself found difficulties in applying his own theory without reservation to many cases and therefore found it necessary to bolster up his theory with subsidiary hypotheses of doubtful tenability. I refer particularly to his theory of germinal selection (1902) which was devised to explain away any resemblances to use-or-disuse-inheritance in a manner consistent with his general theory of the continuity and independence of the germ-plasm. Spencer had argued that the natural selection of minute differences was insufficient to explain the partial or complete loss of appendages such as the pelvic in the whale (quoted from Kellogg, 1907). What possible preference could natural selection exert in a struggle for existence if chance germinal variations left more than the merest vestige or more than complete obliteration of functionless parts? In the whale, the femur weighs at most a few ounces, and is entirely concealed in a mass of flesh. Spencer maintained it was absurd to attribute such an enormous reduction of the pelvic appendage to natural selection; for it was inconceivable that any whales would be eliminated by natural selection in competition with other whales, if the vestigial appendage weighed perhaps a few pounds instead of a few ounces. Such objections led both Roux and Weismann to formulate their respective theories of "battle of the parts" and "the theory of germinal selection." Roux (1881) assumed that the sheer functional activity of an organ acted as a stimulus which led to its development at the expense of other less used or useless organs; but Plate (1903) pointed out that Roux's hypothesis really implied the inheritance of the effects of use and disuse. Weismann (1902) assumed a similar sort of struggle between the determinants in the germ-plasm for the available nutrition, in which struggle the stronger determinants were perpetuated and grew at the expense of weaker ones. Now this starvation of determinants leading

to the degeneration or loss of the organs for which the determinants are responsible is a pure assumption, and in this respect has no advantage over the Lamarckian hypothesis. No one has observed such germinal selection; nor is it even categorically imperative to assume that the actual observable phenomena *must* be causally connected with such hypothesized germinal selection, to the exclusion of any other possible antecedent condition which might conceivably modify germ-plasm to produce racial adaptation.

The citation of similar cases and the arguments could be carried on *ad infinitum et ad nauseam usque*. The subject has been discussed from partisan and from impartial viewpoints and excellent bibliographies have been prepared by Kellogg (1907), Przibram (1910), Thomson (1910), Semon (1912), Kammerer (1924) and others.

The evidence from a direct examination of the germ-cells as revealed in cytological and embryological studies, is often cited as bearing on our question. In the maw-worm (Ascaris megalocephala) from the stomach of the horse, it has been shown that the two cells of the second cleavage mitosis are demonstrably different. The integrity of the chromosomes of one blastomere stands out in contrast to the diminution of chromatin material in the second blastomere. In the third and fourth cleavage mitosis, but one blastomere persists in which the chromosomes are entire. In the fifth cleavage mitosis, one undiminished embryonic cell becomes the primordial germ cell and gives rise to the future germ cells. Somewhat similar conditions are seen in some other Ascarids (parasitic Nematodes), in the fly Miastor, and a few other forms. Such striking instances are few, but they are often seized upon as fully sufficient evidence bearing on the continuity and independence of the germ-plasm. However the distinction between body and germ cells is less emphasized at the present day and cannot be drawn as sharply as we were often inclined to state in elementary text-books for pedagogic reasons. In the first place there is no such distinction between germ-plasm and body in the unicellular forms, in some lower animals, and in plants; and secondly, there may be no such distinction in many higher animals. Hargitt (1913), (1919) for example showed that in Campanularia (one of the Coelenterates) the basal portion of a dividing entodermal cell might form an egg, but the distal portion remain as functional epithelium. Hargitt also reports (1924) that the germ cells in the amphibian, Diemyctylus viridescens, may be at times derived from peritoneal epithelium. Janda (1912) removed completely the sex gland in Criodrilus (one of the Oligochaetes) by

simply cutting off sufficient anterior segments. New gonads were formed from the peritoneal cells of the septum. The production of germ cells is then a problem of differentiation and dedifferentiation as Child (1915) and others have sugggested. Now the continuity and independence of the germ-plasm were often represented as associated self-consistent elements adding to the plausibility of the Weismannian hypothesis; but it is safe to say that the continuity of the germ-plasm is an insecure element in the argument, and moreover has no imperative connection with its independence. These are separate questions. Regardless of whether the germ-plasm is a continuous unbroken stream from generation to generation or whether there is an interposition of somatic cells, its origin does not assure immunity from all external influences affecting the body, nor does it provide for susceptibility to such influences. Its origin plays no part either way. A more detailed statement of the relation of cytology to the question in hand, together with adequate citations, is given by Sharpe (1922).

All of the foregoing discussion has dealt with the suggestive or circumstantial evidence, which is frankly acknowledged to be indirect. In simple situations, conclusions may be drawn from this kind of evidence with a reasonable degree of certainty; but when we are dealing with exceedingly complex biological conditions, we cannot be other than extremely cautious and conservative. Phylogenetic degeneration of organs and disuse may go together, and progressive racial development may accompany increased use; but which is cause and which is effect, it is impossible to say with certainty. We may even present strong presumptive evidence as in Duerden's ostrich studies; however we do not *prove* the inheritance of an acquired character and we must usually admit the possibility of several alternative explanations. If we possessed all the facts, the solution might then disclose some things no one ever happened to think of before,—an event not altogether novel in the annals of biological history. Such a situation is unsatisfactory. Consistent with the spirit of investigation, the matter has been subjected to experimental tests.

B. The experimental evidence. The relationships of environment, soma and germ cells are simple and can be made clear at once:

1. The environment may induce slight or radical somatic modifications without any visible effect on the germ cells.

2. The environment may effect germinal changes without any visible somatic response.

3. The environment may modify both soma and germ-plasm simul-

taneously. Whenever the characters of the offspring show that both somatic and germinal modifications were of a corresponding and equivalent type, the result is interpreted as "parallel induction."

4. The environment may first modify the soma and this effect may then be transferred to the germ-cells. This hypothetical situation is called "somatic induction."

For the sake of convenience in discussion I shall consider the experimental data under these divisions.

1. Direct somatic response to environment. Perhaps most influences brought to bear on the soma under experimental conditions show no evidence of their effects in subsequent generations after the removal of the original stimulus. Certainly mutilations such as Weismann (1893) performed when removing the tails of young mice for nineteen consecutive generations were without hereditary effects, and these results have been fully corroborated by others (Cope, Bos, Mantegazza and Rosenthal, quoted from Thomson (1913) p. 224). It is commonplace knowledge that docking dogs and sheep, dehorning cattle, cropping the ears of dogs, circumcision, and similar mutilations are without hereditary effects.

Roberts (1918) exposed a race of fruit-flies (Drosophila melanogaster) with vestigeal wings to high temperatures. The vestiges were greatly enlarged, giving in some cases apparently fully developed, normal, functional wings. The somatic change induced by a change in environment was however purely temporary; for under normal temperatures the descendants reverted to the ancestral condition.

Perhaps one of the most striking experiments planned to change the soma of a given germ-plasm is found in Castle and Phillips' (1911) ovarian transplantations in guinea pigs. The investigators fastened ovaries from two young black guinea pigs to the tip of the uterine horn of an albino female after her own ovaries had been removed. The "white" soma with "black" ovaries was then mated to a normal albino male and gave rise to six young between the sixth and the twelfth month following the operation. All six young were *black*, which was exactly the same result that would have followed had the albino male been mated to the original black females from which the transplanted ovarian tissue was obtained. In other words, a completely new "white soma" had no perceptible effect on the "black germ-plasm." These ingenious experiments are sometimes cited as proof of the complete independence of the germ-plasm; but we frankly question whether such optimism is justified. The black germ-plasm had been surrounded by a "black

soma" and was then placed in a new "white soma." The only demonstrable difference between the two somas was the genetic factor for color. In fact it seems reasonable to believe there could have been but few other marked differences at most, for autoplastic transplantations are much more likely to be successful than heteroplastic. We hardly dare conclude *any* or *all* somatic changes can never affect the germ-plasm simply because in these experiments a simple recessive factor for albinism in the new white soma failed to modify a single dominant factor for color in the germ-plasm. In fact, as far as we know, albinism may indeed be due to the absence of an enzyme, and an enzyme absent in somatic cells could hardly be expected to modify germ-plasm.

It is futile to list all cases of experimentally induced somatic response, since these constitute only a negative sort of evidence and have been recorded and discussed by Kellogg (1907), Przibram (1911), Thomson (1912) and others. Hardly anyone now questions that mutilations are not inherited as such; furthermore it is freely admitted that simple or radical somatic changes have been tolerated without demonstrable germinal effects. All such direct somatic changes without germinal effects do not exclude the possibility that some kinds of somatic changes may have germinal effects.

2. Direct germinal response to environment. It is perfectly clear that the soma may be easily modified without producing hereditary effects. The reverse is also possible, for the germ-cells may be affected directly by temperature, x-rays, alcohol and other physical and chemical agents and produce hereditary effects without first modifying the soma. The action of these agents on the germ-cells may be indifferent, selective, harmful or lethal, and give rise to simple changes in color or color-pattern, or to pronounced changes in the growth-rate and the normal development.

Tower (1906) reported pronounced effects of environment (variations in temperature and humidity) upon the color and color-pattern of the potato-beetle, Leptinotarsa. If the stimulus was applied to the eggs or larvae little or no later change was apparent; but if the stimulus was applied to the pupae, unmistakable color-changes appeared in the imago or adult which were not inherited. When however adults were subjected to the same experimental conditions during the so-called sensitive period of germ-cell maturation the progeny alone were modified, and these modifications were inherited. The presumptive evidence would indicate a direct response of the germ-plasm in the latter case.

Among mammals, cases of germinal modification are also reported. Stockard and Papanicolau (1916), (1918) subjected guinea pigs to alco-

hol fumes; and while the immediate subjects were not injured, the offspring and later generations showed a record of less fertility, greater mortality, arrested development, and defects of the central nervous system and of the eyes. Cole and Bachhuber (1914) fed male rabbits and fowls lead acetate and concluded there was a definite and demonstrable effect on the progeny as shown by their retarded development. Recently Little and Bagg (1924) reported on the appearance of hereditary structural defects in the F_2 and later descendants of mice exposed to light doses of x-rays. The defects included eye defects, club-feet, extreme brachycephaly, etc. In inheritance these defects acted in a general way like Mendelian recessives, although there was much variability in the amount and kind of defectiveness shown. The investigators are conservative but intimate they have some grounds for believing that these abnormalities were cases of direct germinal response, related to the x-ray treatment. Some of these defects are found in mice not treated with x-rays.

There should be no confusion in these cases of direct germinal response, for none of them represents in any sense the inheritance of an acquired somatic modification.

3. Parallel induction. If either the soma or the germ cells may be modified by environmental stimuli, as we have seen, we may expect to find cases where both soma and germ cells are affected simultaneously. Whenever the nature of the somatic modification has no clear resemblance to, or relation to the germinal modification as shown by the offspring, there can be no question of the inheritance of an acquired somatic response. For example, Stockard and Papanicolau (1916) treated guinea pigs with alcohol fumes and observed that the treatment produced corneal irritation and opacity in some cases; but the effect on the germ cells was *not* such as to reproduce the parental modification. The pronounced germinal effect as described above was qualitatively and quantitatively independent of this slight somatic response. There was no question of *parallel* induction or of the inheritance of an acquired character. In contrast to Stockard's case, a few experiments have been recorded which are sometimes interpreted as cases of parallel induction, which denotes that both soma and germ cells are independently modified by the same environmental stimulus, but always in such a specific manner that the modified germ-plasm gives rise to a somatic character resembling the original somatic modification. Most frequently cited are the experiments of Standfuss, Fischer, Schröder and Tower.

Standfuss (1898) subjected the pupae of Vanessa urticae (related to our common mourning-cloak butterfly) to freezing temperatures, and melanic variations appeared in the adults. Under normal temperature, seven pairs of these melanic adults gave normal offspring, but four of the male offspring from an eighth pair were again abnormally dark.

Fischer (1901), (1907) performed a similar experiment on Arctia caja, the tiger-moth. A number of pupae were divided into two groups; one kept under normal conditions, and the other exposed to low temperature (8°C.). The adults emerging from the latter group were abnormally dark, the males being more aberrant than the females. Wing and foot abnormalities were also present. Fischer succeeded in obtaining offspring under normal temperature conditions (173 pupae) from the darkest male and female. The first adults to emerge were normal, but among the last to emerge there were 17 melanic individuals (the males were again darker than the females). The wing and foot abnormalities were again present. Thomson (1913) and Castle (1923) suggested pertinently that these moths vary in darkness of coloration under normal conditions and that the experimental treatment merely helped to reveal which animals carried a greater inherent potency for melanism. This enabled Fischer to breed from the widest variates. In other words, Fischer's results were due to selection in which the wider germinal variates were more easily recognized under these special experimental conditions. I am inclined to believe that there is more to Fischer's case than the mere selection of wide variates, and that the retarded ecdysis of the melanic forms and the persistent wing and foot abnormalities suggest some general germinal disturbance which arrested development. It has been suggested that the melanic forms really represent the restoration of an atavistic trait rather than the production of a new character; for von Schuckmann (1909) found the normal untreated chrysalis to be more heavily pigmented than the adult stage.

Schröder (1903) subjected pupae of Abraxas grossulariata (the currant moth) to high temperatures. Here again melanism was produced, and the males were darker than the females. The offspring of the modified adults were in part normal and in part melanic although they were reared under normal conditions.

Tower's experiments (1906) on the potato beetle have already been mentioned in conjunction with direct germinal response. In these experiments he was also apparently able to regulate the stimulus so as to dissociate the somatic from the germinal effect. When variations of temperature and humidity were brought to play upon the metamor-

phosing pupae, the modifications in the emerging adult were not transmitted, but when the adults were subjected to the same experimental conditions during the so-called sensitive period of germ cell maturation the progeny alone were modified and the modifications were inherited. Now when the stimulus was applied to both the pupal and adult stages, both the somatic and germinal changes took place, so that the modified beetle gave rise to modified progeny, and the case appeared like the inheritance of an acquired somatic modification. The logical interpretation is suggested from the first two types of experiments, and it would seem to be a case of parallel induction, or similar effects of environment on both soma and germ cells. Many objections to Tower's work have been raised, some of them relating to the accuracy and validity of the data. The work has not been repeated but the conclusions and implications have such an important bearing on the question of evolutionary changes as determined directly by environment that it would be worth while repeating. Until that time we shall have to hold a final decision on these experiments in abeyance.

Altogether, this array of experiments which might be interpreted as cases of "parallel induction" is not imposing. Furthermore it is still an open question whether this interpretation is the correct one. Weismann, however, was sufficiently impressed so that he felt it necessary to invoke this subsidiary hypothesis of parallel induction; for naturally, having once denied that somatic effects may be registered on the germplasm, it was necessary to conclude that both soma and germ cells were directly, but independently, affected by the environmental stimulus. We have no real knowledge as to just how parallel induction could take place if it actually does. The general thought at the bottom of any explanation for this hypothesized parallel induction is as follows: there *may perhaps* (we do not know that there is) be enough chemical and physical similarity between the somatic character and its Anlagen or determiners in the germ cells so that when a stimulus calls forth a somatic response, it also effects a correlated and corresponding change in the germ cells. These modified germ cells are then supposed to give rise to a somatic character in subsequent generations which shall resemble to some extent at least the somatic response acquired under the initial stimulus. All of this has the earmarks of an involved compromise and clever device to save the integrity of the original premise, the independence of the germ-plasm. Parallel induction is certainly not proven in the Standfuss, Fischer and Schröder experiments. However, the *hypothesis* has attracted a number of biologists because it offers

a possible explanation for evolutionary change depending on germinal changes, and yet running parallel to somatic modification without being produced by the somatic modification. Hence it does no violence to Weismann's hypothesis of germinal independence and, at the same time, it shows how the Lamarckian principle may operate when certain types of bodily changes are presumably produced by environment and reappear in the progeny. Indeed some biologists are inclined to look upon parallel induction as tantamount to a fatal admission by the Weismannians that some sort of Lamarckian explanation is necessary after all. The very meagerness of experimental data alone, having any bearing on this question, should make us hesitate to announce at present any far-reaching generalities as to the importance of parallel induction. Semon (1911), (1912), one of the recent ardent advocates of Lamarckian persuasion, is not inclined to attribute more than a very minor rôle, if any, to parallel induction. Further critical and enlarged investigations are necessary and desirable in this whole field.

4. Somatic induction. A number of experiments have been recorded which were at the time not easily classified as cases of direct germinal response or as cases of parallel induction. Later and more complete information has enabled us to see some of these cases in their true light. There still remains a group of experiments which, as they stand, are interpreted by some as cases of somatic induction. By this we mean that the somatic response appears *first*, and its effects are *then* transferred to the germ-plasm in such a manner as to cause the reappearance of the somatic modification, at least in part, in the offspring after the original stimulus has been removed.

For many years Brown-Sequard's experiments were prominently quoted in almost every discussion of this subject. Partial or complete section of or injury to spinal cord, sciatic nerve or central nervous system in guinea pigs was followed by epileptoid symptoms, various morbid states of the nervous system, eye-defects, etc. In many cases the offspring showed similar abnormalties. Brown-Sequard's results were repeated and confirmed by Westphal, Obersteiner, Romanes and others (quoted from Semon (1911) and Thomson (1910)); but Sommer (1900) failed to confirm them. However, it should be stated that Sommer used a very small number of animals. The evidence in the Brown-Sequard experiments has been variously received (or neglected), depending largely on the predilections of the critic. In general, one is impressed by the efforts made to explain them away by several different routes. This can only be taken to mean that the inferences are not

clear and consistent. It is pointed out that the guinea pig is in any event predisposed to epileptoid symptoms brought on by stimulating an epileptogenic area, perhaps in scratching itself. Furthermore the results may be due in part to infection, or due to toxins or specific cytotoxins set free in the original lesion, or due in part to "pure coincidence." There is always some danger of abusing this last explanation. (For a complete bibliography of Brown-Sequard's numerous papers see Maciesza and Wrzosek (1911).)

Schröder (1903) reported experiments of an entirely different nature. The caterpillar of the willow moth (Gracilaria stigmatella) has a peculiar habit of enclosing itself by rolling over the free end of the leaf and binding it down with its web. By cutting off the ends of the leaves, Schröder forced them to roll over either one or both sides of the leaves in 84 out of 91 cases. Under the same experimental conditions all of the 43 offspring showed a similar adaptation. In the third generation, 19 larvae were given the normal uncut leaves and 4 of them persisted in showing the modified habit. Schröder also attempted another experiment to modify instinctive bahavior. The larvae of the willow-beetle (Phratora vitellinae) which lives on a smooth-leaved variety according to Schröder (1903) were placed on a pubescent type. The adults showed a very great preference for the original smooth-leaf variety; but such eggs as were laid on the smooth-leaf plant were removed to the pubescent type. Repeating this process for three generations, Schröder claims to have gradually developed a condition where the new pubescent food-plant was adopted. There are details in Schröder's experiments on which additional information would be desirable. As far as they have proceeded, they are suggestive; but we should like to know 1, how much variability in instinct under normal conditions these insects would show if very large numbers were examined; and 2, whether the modifications would persist or disappear gradually if a reasonable number of additional generations were followed under normal conditions. Furthermore, the actual number of cases of modification was rather small and larger numbers would be more convincing. It is difficult to understand why Schröder was content with a relatively small number of subjects and apparently discontinued his experiments when the results were so suggestive and had such a grave bearing on an important disputed theory.

Perhaps no investigator has been more active or more frequently quoted in recent years than Kammerer (1911), (1924). Although his experiments are numerous, I shall refer in detail to only three as fairly

typical. Kammerer (1924) reviews his numerous experiments and gives a detailed bibliography of his work in his recent book. While, no doubt, Kammerer meant to be as fair-minded in his book as anyone can be who holds a decidedly partisan view on a debated question, his résumé of the subject leaves one with the impression that his interpretations are more casuistic than penetrating, and that his enthusiasm for facts has given way to a premature zealous endeavor to convert the unbeliever.

In a number of his experiments with amphibians, Kammerer made frequent use of the facility with which adaptive changes may be induced in two respects, viz.: in bodily color, and in habits of reproduction. For example, the European salamander (Salamandra maculosa) has irregular and variable yellow spots on a black body. Kammerer kept for several years the darker variates on a yellow background, and the yellower variates on a dark background. He chose his materials in this way to avoid the criticism that the original chosen forms might already vary in the direction of adaptation to the proposed treatment. In the first experiment he found a marked extension of the yellow body-color, and in the second a marked extension in black body-color, i.e., well-known individual adaptations for concealment. In the first experiment Kammerer divided the offspring of animals with extended yellow areas into two groups. One group reared on a yellow background now developed *inordinately* extended yellow areas. The other group reared on a black background showed less yellow, but nevertheless more yellow than the normal salamander, and Kammerer claims (his figures also indicate it) much yellow considering the expected effect of a black background. Analogous breeding experiments gave equivalent results when the original parent forms were kept on the black background. How are we to explain this ostensible persistence of a parental modification? Šećerov (1912) placed capsules containing photosensitive paper within the body cavity of the salamander; and finding that $\frac{1}{173}$ of the total impinging light penetrated to the gonads, he suggested the possibility of parallel induction. Castle (1923) attributed Kammerer's results to simple selection; for under the same given yellow environment, those animals which appeared to be most sensitive to the treatment were the very ones which had the greatest genetic potentiality for yellow. Environment merely brought it out. Breeding from these and rearing the offspring in part on a black and in part on a yellow background would naturally give animals capable of producing enlarged

yellow areas. The environmental influences on the soma are not denied; but they are superimposed on the genetic potentialities. Castle's suggestion sounds reasonable and should be put to the obvious experimental test.

This same black and yellow salamander (Salamandra maculosa) which inhabits damp woods is viviparous or ovo-viviparous both in its natural habitat and in captivity. The large number of young, already furnished with gills, is deposited in the water; and after several months they undergo complete metamorphosis, preparatory to terrestrial life. Salamandra atra, a closely related black Alpine species, is viviparous, giving birth to two young, completely metamorphosed and immediately adapted to terrestrial life. Kammerer sought to change the former form into the latter by keeping it from water. This black and yellow salamander, Salamandra maculosa, was thus forced to retain its embryos in the uterus until they were completely metamorphosed, and ready for terrestrial life as in the case of the black salamander. In several other respects the black and yellow salamander began to resemble the black Alpine species, viz.: 1, number of young at birth was greatly reduced (2 to 7 offspring); 2, the intra-uterine fate of eggs and embryos approached that of the black salamander; and 3, the color of the young was almost totally black. These animals thus produced under experimental conditions were given access to water when sexually mature. They deposited their young in the water in an advanced stage of metamorphosis, leaving the water in a few days rather than in a few months. The reverse of these foregoing experiments was tried on the black Alpine species with analogous results; thus there was a partial persistence of an acquired mode of reproduction. Thomson (1913) raised some questions relative to all these experiments. He suggested that "they do not deal with a structural modification in the ordinary sense;" but after all what of it? Furthermore "it may be that the experimental conditions acted directly on the germ cells of the original subjects of experiment." This is perhaps remotely possible or conceivable, but it remains to be demonstrated and elucidated in detail.

In our present consideration of this general subject, we are concerned primarily with the bare facts of Kammerer's experiments and not with his excursive and premature ideas on their future application to the welfare of mankind, which on the whole have rather alienated the attention of the more serious worker from the really important feature of Kammerer's work, namely, the experimental data. From the point of view of one attempting to survey and evaluate the *bare facts* in an

objective way and to weigh the arguments and criticisms advanced by both Lamarckian and Weismannian fraternities, the variety of opinions, attitudes and reactions toward Kammerer's experiments is at first confusing. Some, having looked upon the prime question as clearly settled, either neglect Kammerer's work or handle it very gingerly. Others are inclined to offer such objections as the inadvertent selection of materials during the course of the experiment, direct germinal response with or without coincident somatic modification, records too meager to warrant conclusions, corroborative experiments lacking, and the like. And yet still others accept the data and their implications at face value without hesitation and sometimes rather uncritically. One and the same experiment may elicit censure from one critic and approval from another. For example, Kammerer (1924) cut off the siphons of Ciona intestinalis (a soft bodied marine animal, Ascidian) and found as others had that new siphons of abnormal length were regenerated. Kammerer claimed the offspring also developed abnormally long siphons. One critic suggests these experiments are the only tangible bit of real evidence Kammerer has advanced. Another says, "In view of all the negative evidence furnished by other animals this case, as yet incompletely published, seems highly improbable. The unsupported claim throws more light upon the credibility of Kammerer as a witness (and he has brought forward many cases in recent years) than upon the general question of the inheritance of mutilations." Incidentally it should be pointed out that no claim was made for the inheritance of a mutilation; but rather a stimulus to growth produced by the mutilation has persisted, if Kammerer's contentions are well founded. We shall make little if any progress by comparing the predilections of the critics. I frankly confess we know very little of the real and complete explanation back of many of Kammerer's results. In many cases Kammerer's experiments and records end abruptly where, it seems to me, the minutest details are most essential. Nevertheless, we must credit Kammerer with much suggestive material and with an effort in a desirable direction,—desirable because the larger question of hereditary adaptations has not been cogently and adequately answered. There have been without doubt many carelessly claimed and unfounded instances of the apparent transmission of parental modifications, in fact so many that one has a right to be more than usually skeptical and to demand rigid proof. Mere skepticism, however, does not help to solve this major question of adaptive evolution.

One of the chief objections raised by the Weismannians is that we

know of no mechanism whereby parental modifications may be transmitted; and the Lamarckians have admitted this, but have contented themselves by pointing out that our lack of knowledge is not a good argument against the possibility of such a mechanism. From time to time some suggestions have been made as to how such transmission might be accomplished, but anything resembling complete proof has been lacking. Charrin (1896) maintained long ago that toxins of the mother might affect the fetus, and toxins (or cytotoxins, Thomson (1913)) in either sex might affect the germ cells directly.

More recently Guyer and Smith (1918), 1920), (1921), (1922), (1923) have presented evidence which may prove to point in the same direction. They made an emulsion of rabbit lens and injected it into fowl. It has been long known that such a foreign protein acts as an antigen and the host forms antibodies as a characteristic protective reaction. The fowl was therefore a source of a cytolytic serum which breaks down rabbit lens substance. When Guyer and Smith injected this fowl serum into pregnant rabbits (at about the 10th to 14th day of pregnancy) there was no effect on the adult, but several young had unmistakable eye-defects. The presumption is that the lens antibodies had no effect on the adult lens because it was already cut off from the general blood circulation; but since the embryo lens was surrounded by a rich vascular tissue, the specific cytolysins reached and broke down fetal lens substance. Of 61 surviving offspring from treated pregnant females 4 had one or both eyes conspicuously defective and 5 had eyes clearly abnormal. The conspicuously defective were the offspring of 2 different females. The defects were of various types: lens-opacity, cleft iris, persistent hyaloid artery, microphthalmia, complete disappearance of the eyeball and the like. In none of several hundred controls (including animals treated with other lytic sera) did abnormal eyes appear. The outstanding feature of these experiments is that with no further treatment the defective-eyed condition has been transmitted even through the present ninth generation. To assure inheritance in the true sense (rather than the possible transmission of antibodies *via* the placenta) the defect has repeatedly been transmitted through the male. When defective males were mated to normal females of unrelated strains, the F_1 hybrids were normal and the defects reappeared in the F_2 generation. In general, the defects behave roughly like Mendelian recessives, although they are evidently neither simple nor regular.

The importance of these experimental facts as indicating a possible

effective means of producing germinal changes through bodily modifications is contingent on the possibility of producing specific auto-antibodies by a change in the animal tissues through environment. Will an autolysis of lens substance (or other tissues) *in situ* and brought about by the usual environmental forces activate the production of specific autocytotoxins and auto-antibodies? Guyer also injected pulped rabbit-lens intravenously into 11 rabbits just before or during pregnancy, and obtained from one female (the 84 line) hereditary defects of the same nature as those found following the use of fowl serum immunized to rabbit lens. Furthermore he reported that the normal rabbit developed lens antibodies in its blood serum when its lens was injured, for in 8 cases lens precipitins developed after the lens had been broken up *in situ* by needling.[4]

It is still an open and debated question whether Guyer's experiments represent parallel induction or somatic induction or neither. If in the original experiments the lens antibodies attacked the fetal lens and some of its germinal correlatives simultaneously then we must place the results in the category of parallel induction; but if an injured lens can itself develop auto-antibodies which then attack the germinal correlatives, we have a case of somatic induction. It may turn out that both are possible, depending on the conditions of the experiment and method of procedure; for Guyer found, as stated above, lens precipitins in the blood stream after needling the lens of normal rabbits, and also in the blood serum of defective-eyed individuals whose defects were hereditary. Furthermore Guyer stated that "since without any subsequent treatments the defects became more and more pronounced in successive generations, the degenerating eyes might themselves be directly or indirectly originating antibodies or other chemical substances in the bloodstream of their bearer which in turn affect the germ cells. These once established, should be as effective in modifying germinal factors as corresponding antibodies introduced into the fetus through the placenta of the mother." No doubt a germinal change took place, because normal rabbits carrying the defect reproduced it when mated inter se. Stockard (1923) suggested pertinently that this whole group of eye-defects might be the result of the highly toxic lens antigen which acted like other well known toxic agents and simply injured the germ cells in

[4] In his recent presidential address before American Society of Zoölogists, Guyer reported defective-eyed young from such subjects. He cautiously stated that such defective-eyed young had not yet been bred to demonstrate the hereditary nature of the defects.

a general way so as to retard development, the eye being affected because it was a center of high metabolic activity, rather than because the lens antigen was specific in its action; for, said Stockard, "If the reaction to the lens substances was specific, both lenses of the embryo should be affected and not only one as is often the case, and the degeneracy of the eye ball and retina would not be expected." Guyer, however, has stated that other lytic sera (in the case of spermatotoxins) do not affect the eye. I am not sure that specificity and perfect bilateral symmetry are necessarily associated for we find definitely inherited genes which affect definite structures but the effect is not infrequently asymmetrical and variable; as for example, in polydactylism and spotting in guinea pigs.

Szily (1924), in his detailed paper on eye-defects written from the viewpoint of the ophthalmologist, takes issue with Guyer and Smith and fails to see any evidence for the experimental modification of germplasm. His strictures relate chiefly to the following:

1. Hereditary eye-defects are well known in rabbits, and occur without previous treatment. Von Hippel, von Szily, Hochstetter and Koyanagi have recorded such cases, and were able to breed numerous similarly defective offspring. The detailed references are given by Szily (1924). Had Guyer and Smith known of these cases, they would have been more conservative.

2. Szily implies that the normal rabbit, ♂ 2, sire of ♂ 3A 1, from which a long line of defective-eyed was derived, was a heterozygous carrier of eye defects. Szily thinks it entirely probable that ♀ 16A1, the progenitor of another long line of defects was a descendant of the original ♂ 2, even though her dam was the daughter of a female unrelated to the Madison stock, as Guyer and Smith state.

3. Silfvast (1922) and Findlay (1924) attempted similar (but not identical) experiments with negative results.

Szily's objections do not however peremptorily dispose of Guyer's case. In justice to Guyer it should be stated that only two of these four hereditary cases which Szily cites were published before Guyer's work began, and while these might be familiar to the ophthalmologist, they might well escape the experimental zoölogist. In any event Guyer (1920) recognized the possibility of a chance hereditary defect being carried in his original stocks and stated "Since, in our opinion, the fact of transmissibility is by far the most significant one established, in repeating the original experiment we have taken pains to secure wholly unrelated stock so that we may be sure that we are not simply

perpetuating a chance inheritable defect which has sprung up by some strange coincidence just at the time of our work. To safeguard the experiments in this respect, we have imported rabbits from other States (Minnesota, Iowa, Illinois and Indiana) and tested them genetically before treating them with serum or crossing them with our original strain." It is true that Guyer did regard rabbits as "stabile forms wholly unlikely to develop eye-defects unless, as in our work, these have been deliberately induced by the experimenter." Since we now know that similar eye-defects occur without experimental treatment the whole question boils down to one of probabilities; and instead of regarding it as "wholly unlikely" we must inquire how probable it is that Guyer accidentally chose defect-carriers as subjects for experimental treatment. If it could be demonstrated that Guyer's 16A 1 line was genetically related to ♂ 2, it would naturally arouse our skepticism as to the original cause of these eye-defects. However, Szily has not demonstrated that these lines are related, even though it is convenient for his exposition to suspect that they may be. Now when Guyer and Smith state explicitly (1920, p. 186) that "Rabbit 16A 1 was the daughter of a female shipped from Minneapolis and was, therefore, unrelated to our Madison stock," I cannot as yet conclude that they naïvely overlook the possibility of relationship through ♂ 2, if that were the case. We have neither data nor theory, which we may use as the basis for an experimental or à priori probability, and it is therefore difficult to say *how* likely one is to chance upon a normal rabbit which carries latent germinal factors for eye-defects. If the 3A 1, and 16A 1, the 84, and the New Zealand Red lines (in this last line sheep serum immunized to rabbit lens was employed and eye defects were shown only in the fetal young), in all of which the experimental treatment was followed by eye defects, prove to be genetically unrelated then it is not probable that Guyer chanced upon separate stocks carrying latent hereditary defects which would have cropped out anyway regardless of treatment[5]

While Silfvast and Findlay could not duplicate Guyer and Smith's results in similar experiments, it still remains true that their experiments were not identical with Guyer and Smith's. Silfvast experimented with rabbits using pig-lens emulsion for both active and pas-

[5] In response to my inquiry, Professor Guyer kindly informed me, that ♀ 16 A1 and ♀ 84 were daughters of pregnant females from Minneapolis, Minn., and Lafayette, Ind., respectively. They were therefore in all probability unrelated to each other or to ♂ 2 and the 3 A1 line. This information has a decided bearing on Szily's implications.

sive immunization. Findlay used rat, sheep and ox lens as antigens in active and passive immunization of rats and mice. For the time being the entire group of results which Guyer reports must be looked upon as suggestive, and final decision delayed until more facts have been accumulated, and legitimate objections reconciled. The whole problem of any organ specific action of antibodies is still under discussion (see Szily, 1924, p. 30).

The mere reappearance of a modification in several consecutive generations is sometimes confused with true inheritance, but the two are not necessarily identical because the reappearance may be due to the persistence of an unusual condition or an obscure environmental stimulus extending over a series of generations. Thus an acquired modification due to an obscure infection may reappear in the progeny simply because they are reinfected rather than because the acquired modification has been inherited. The cases of persistent vestibular defects reported by Griffith (1922) now appear to me to be explicable *in part* on this basis. Griffith subjected albino rats to long periods of continuous rotation (up to one and a half years) by keeping them in circular pens turning at 60 and 90 R.P.M., some in a clockwise and others in a counter-clockwise direction. Several weeks after removal from the rotating pens, a number of subjects developed a specific type of disequilibration, showing among other effects a peculiar twist or tilt of the head and permanent modifications in ocular after-nystagmus. Now in the normal rat, the ocular oscillations which follow a unit stimulus of 20 turns in 10 inches on the cyclostat (in either a clockwise or counter-clockwise direction) lasts about 10 seconds for animals over six months old. It varies inversely with age as I have since found, the greatest change occurring within the earlier months of life. (At 30 days it lasts about 13 seconds.) The disequilibration which Griffith reported was specific inasmuch as long-continued clockwise rotation was followed by a left twist of the head and by a very much reduced ocular after-nystagmus when the unit cyclostat stimulus was in a clockwise direction; but after-nystagmus was normal when tested by a counter-clockwise unit stimulus. Long continued counter-clockwise rotation gave the exact reverse picture. Putting these observations in tabular form in order to visualize and compare them easily, we have approximately the following:

Character of ocular after-nystagmus

DIRECTION OF UNIT CYCLOSTAT STIMULUS	WITH LEFT HEAD-TWIST FOLLOWING LONG CLOCK-WISE ROTATION	WITH RIGHT HEAD-TWIST FOLLOWING LONG COUNTER-CLOCKWISE ROTATION
Clockwise	Reduced to about one-half normal	Normal
Counter-clockwise	Normal	Reduced to about one-half normal

The permanently reduced clockwise after-nystagmus following long continued clockwise rotation and the analogous results from long continued counter-clockwise rotation thus gave the appearance of a permanent habituation. Furthermore Griffith claimed the progeny frequently showed similar cases of disequilibration, always specific and "regularly dependent upon the direction in which the ancestors were rotated." No such subjects appeared in Griffith's large related control stock. Similar disequilibrated rats do occur, however, without any previous rotational history as shown by Cassamajor (1914), Detlefsen (1923), and McCordock and Congdon (1924).

The obscure point in Griffith's findings that may have bearing on the question of inheritance of acquired somatic characters is the specific nature of the disequilibration. I was able to test for after-nystagmus 72 cases of middle-ear infection in a colony of some 6000 rats. Of these 72, 37 showed a right twist of the head, and 35 a left twist of the head. (The tilt of the head is toward the side showing the lesion.) As far as external appearances (posture, head twist, position of eyes, etc.) or modifications of normal after-nystagmus were concerned, all of those with the occiput turned to either the left or the right, as the case might be, were indistinguishable from Griffith's animals with a left head twist following clockwise rotation, or a right head twist following counter-clockwise rotation, respectively. On the basis of 986 cyclostat tests on these 72 cases the following table was prepared, giving the means and standard deviations for the length of ocular after-nystagmus in seconds following a unit cyclostat stimulus:

DIRECTION OF UNIT-STIMULUS	AVERAGE LENGTH OF OCULAR AFTER-NYSTAGMUS IN SECONDS	
	Occiput to left (Left labyrinth violated right labyrinth intact)	Occiput to right (Right labyrinth violated left labyrinth intact)
Clockwise	M = 5.36 σ = 1.32	M = 9.61 σ = 2.16
Counter-clockwise	M = 9.56 σ = 2.21	M = 5.35 σ = 1.11

The essential similarity between Griffith's rotated specimens and my cases of labyrinthitis is apparent.

It should be noted at the outset that a left labyrinthitis gives the *reverse* of a right labyrinthitis. Any circumstance which violates the integrity of the left labyrinth gives a reduced clockwise after-nystagmus, and similarly for the right labyrinth a reduced counter-clockwise after-nystagmus. I have inactivated a single labyrinth by intense heat or by burring with a dental burr and have found results qualitatively and quantitatively similar to these cases of a suppurative labyrinthitis. It is therefore reasonably clear that the specific individual habituation of Griffith's *disequilibrated* specimens is purely illusory and does not represent habituation in any real sense, but is dependent upon the function of the individual labyrinth. Perhaps the cyclostat tests in these pathological cases suggest the real reason back of any specificity (positive and complete association between direction of rotation and type of disequilibration) such as Griffith reported in his cases of immediate disequilibration following rotational treatment. If for example the right labyrinth is more sensitive to counter-clockwise rotation in any plane (both with acceleration and retardation), then such long continued counter-clockwise stimulus should first break down the right labyrinth, and leaving the left labyrinth more or less intact would give the illusory appearance of permanent habituation, since the left labyrinth is less sensitive to counter-clockwise rotation. The cyclostat records would be expected to show a marked reduction in length of ocular after-nystagmus from a counter-clockwise stimulus on the cyclostat exactly as they do in my cases of labyrinthitis on the right side. In my own rotation experiments using continuous intermittent rotation in a given direction (rotating one minute and resting one minute) over long periods of time and covering four generations, several such dis-

equilibrated specimens have appeared. Five cases showed an association such as Griffith found between the type of rotation and the nature of the disequilibration, and one did not. While these numbers do not constitute proof in a statistical sense, they are not inconsistent with the foregoing suggestion.

If the permanently disequilibrated cases in Griffith's experiments owe their illusory appearance of permanent habituation to the residual effect left after the inactivation of a single labyrinth accompanied by infection, and if they do not represent even true individual habituation, it is difficult to see how true racial habituation could arise from such antecedents. Griffith reported the continued appearance of specific disequilibration in the non-rotated descendants of rotated ancestors. We may legitimately suspect the transmission of infection by parents to offspring, but it should be stated emphatically that sheer reinfection does not explain the specific nature of disequilibration in the non-rotated progeny ("regularly dependent upon the direction in which the ancestors were rotated"). In this respect Griffith's observations stand unique, and uncorroborated. My own records on the incidence of ear infection show that in a random group about 1 to 2 per cent of our albino rats become disequilibrated and that the chances are even for infection on either side. Many albino rats show pus in the bulla, without having the infection attack the inner ear.

No attempt has been made at an exhaustive list of all reputed cases of somatic induction. In many cases the available data are as yet too meager to justify an estimate of their value, and discussion is futile. In other cases, alternative explanations are apparent. The bibliographies cited above give ample references to these.

SUMMARY

The object of this review has not been to prove or disprove the inheritance of somatic modifications but rather to attempt an impartial estimate and adjudication of conflicting views. It is a foregone conclusion that the extreme Weismannian and extreme Lamarckian will hardly concur with the writer's viewpoint. The whole problem has been fundamental in all discussions of evolutionary theory. For many years biologists felt free to assume the inheritance of somatic modifications,—the assumption was so simple and direct, and withal it seemed reasonable. Undoubtedly, much was taken for granted which could be neither subjected to, nor verified by experiment. Weismann performed a

distinct service by subjecting the whole situation to a critical examination. The result was a readjustment in our viewpoint, a refusal to accept the Lamarckian interpretation of evolution, and a stimulus to experiment and research. Unfortunately perhaps, Weismann also felt called upon to attribute a complicated theoretical structure to the germ-plasm, and it will always be a matter of surprise that such flights of the imagination were given a quasi-sacred status or even serious attention. Much of this has now only a historical interest. The main features of Weismann's theorizing that have persisted are the general ideas of the continuity and independence of the germ-plasm.

The number of cases where anything resembling a clear cytological demonstration of the continuity of germ-plasm is small. Some recent papers on regeneration and on the origin of the germ-cells in animals lead us to believe that the problem of gonad formation is like that of any other group of cells, i.e., a problem of degree of differentiation. After all, the continuity of the germ-plasm has no imperative relation to its independence. That the germ-plasm is absolutely independent of environment no one has maintained, for undoubtedly it can be modified directly. But to demonstrate that a somatic modification which involves only body-cells can alter inheritance which concerns germ-cells is quite another matter. Furthermore, the Lamarckian scheme demands that the germ-cells shall be so specifically modified as to produce a new hereditary character resembling the original somatic modification. No group of experiments has as yet demonstrated this possibility beyond the shadow of a doubt. While the Lamarckian may decide that too much has been required for proof, it is only fair to draw attention to the following:

1. Many of the reputed cases of the inheritance of an acquired somatic modification have either been disproven to be such, or have lent themselves to other more acceptable explanations. There have been so many carelessly claimed and unfounded citations of the apparent inheritance of parental somatic modifications, that we are justified in being unusually skeptical and demanding rigid proof.

2. No large group of experiments has been carried far enough to convince numerous impartial conservatives who are ready and willing to accept any part of the Lamarckian principles when sufficient evidence is at hand. Contrary to general opinion, there is no unalterable prejudice in the minds of most students of this question. They simply insist on adequate proof.

3. If somatic induction (or even parallel induction) were a common

or usual modus operandi of evolution, we should perhaps expect to find more positive experimental evidence rather than a few doubtful cases.

If an opinion must be pronounced, we can say without hesitation "insufficient experimental proof as yet;" but there remain the following grounds for regarding this question with tolerant open-mindedness:

1. The suggestive or circumstantial evidence drawn from the data of embryology, paleontology, and ecology still remains just as suggestive, for we have found in natural selection a formal but not completely cogent acceptable explanation for many hereditary adaptations and cases of high specialization.

2. The alacrity with which the strict Weismannian could postulate "parallel induction" in the face of such meager experimental evidence is a fatal admission that some element of Lamarckism is convenient after all, i.e., that evolution is guided directly by environmental change.

3. While no one doubts the effectiveness of natural selection or that its existence is categorically determined by the very nature of things, organic evolution is nevertheless no simple matter but on the contrary an enormously complex process in which natural selection is hardly a cause of variation. The whole concept of evolution has been greatly amplified since its inception by additional important information and points of view derived from intensive biological study, but without any doubt much remains to be discovered.

4. It is conceivable that the experiments devised up to the present time have failed to demonstrate conclusively the inheritance of somatic modifications largely because we have not been able to duplicate the conditions which have been effective over long series of generations in the process of organic evolution.

5. While most biologists have not been fully convinced of somatic induction by such experimental evidences as are available, the fact also remains that the results of a number of experiments have not been fully and adequately "explained away."

6. We have accumulated a huge array of important biological facts, but we have not discovered many general laws. Of first causes in evolution we know next to nothing. When we know more about endocrinology, serology and neurology, then we may hope the inter-relationships between various parts of the organism, the control of development, the basis of adaptive behavior, habit and instinct will be clearer to us. At present, it seems to me more important to recognize the situation than to take a dogmatic position.

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